RELATIONSHIP BETWEEN α-AMYLASE DEGRADATION AND THE STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF LEGUME STARCHES

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NOTE TO USERS

Relationship between α-amylase degradation and the structure and physicochemical properties of legume starches

By

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TABLE OF CONTENTS

Chapter 3: Materials and methods

Chapter 4: Results and discussion

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List of figures

List of tables

List of abbreviations

ALC	Amylose-lipid complex
AFM	Atomic force microscopy
AM	Amylose
AML	Amylose leaching
AMP	Amylopectin
ANOVA	Analysis of variance
BV	Blue value
CL	Chain length
CP/MAS	Cross polarization/magic angle spinning
Da	Dalton
DE	Dextrose equivalent
DMA	Dynamic mechanical analysis
DMSO	Dimethylsulfoxide
DNS	3,5 - dinitrosalicylic acid
DP	Degree of polymerization
DSC	Differential scanning calorimetry
EC	Enzyme code
FACE	Fluorophose-assisted charbohydrate electrophoresis
FT/IR	Fourier transform infrared (spectroscopy)
GPC	Gel permeation chromatography
ΔH	Gelatinization enthalpy
$\Delta H/AP$	Gelatinization enthalpy divided by amylopectin content
IA	Iodine affinity
IBC	Iodine binding capacity
M	Molarity (Molar)

MALDI-MS	Matrix-assisted laser desorption/ionization mass spectrometry
M_w	Weight average molecular weight
NMR	Nuclear magnetic resonance
N	Normality
RC	Relative crystallinity
RDS	Rapid digestible starch
RS	Resistant starch
SAXS	Small angle X-ray scattering
SCLCP	Side-chain liquid-crystalline polymer
SD	Standard deviation
SDS	Slowly digestible starch
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SF	Swelling factor
SGBSS	Starch granule-bound starch synthase
T_c	Conclusion temperature
T_o	Onset temperature
T_p	Peak temperature
TEM	Transmission electron microscopy
v/v	Volume/volume
w/v	Weight/volume
w/w	Weight/weight

Abstract

Starches from different cultivars of black beans, pinto beans, smooth peas, lentils and wrinkled peas were isolated and their composition, physicochemical properties and susceptibility towards porcine pancreatic α-amylase were determined.

The yield of starch ranged from 16.4 to 34.1% on a whole seed basis. The shape of the granules in black bean, pinto bean, smooth pea and lentil varied from round to oval to irregular, while compounds in rounded rosette were observed for wrinkled pea starch. Bound and total lipids ranged from 0.26 to 0.80% and 0.35 to 0.84%, respectively. The total amylose content of black bean, pinto bean, smooth pea and lentil were in the range of 30.5–39.3%, whereas that of wrinkled pea was much higher (78.4%). The percentage of lipid-complexed amylose in native starches ranged from 10.3 to 12.2%. The X-ray diffraction pattern was of the 'B' type in wrinkled pea starch and of the 'C' type in the other starches. The relative crystallinity and the 'B' polymorphic content ranged from 17.7 to 33.4% and 27.1 to 92.2%, respectively. Wrinkled pea starch exhibited the lowest relative crystallinity (17.7%) and the highest 'B' polymorphic content (92.2%).

The swelling factor (SF) and the extent of amylose leaching (AML) of native starches were in the range of 3.4–17.7 and 11.0–17.8%, respectively. The gelatinization temperatures (T_o, $T_{p,}$, T_c) and enthalpy (ΔH) of native starches (with the exception of wrinkled pea starch) were in the range of 60.0–65.7°C, 66.0–76.5°C, 76.4–88.8°C, and 14.6–20.1mJ/mg, respectively. Differences in SF, AML, T_o, T_p, T_c, and ΔH between cultivars of the same species were more pronounced in black bean and lentil starches. Wrinkled pea starch did not show an endothermic peak indicating that starch chain interactions within the amorphous domains were more extensive in wrinkled pea starch.

All starches exhibited a biphasic hydrolysis pattern, i.e a relatively rapid rate initially followed by a progressively decreasing rate thereafter. Wrinkled pea starch exhibited a much higher initial hydrolysis velocity than did the other starches. Cultivars of black bean and lentil showed significant differences in their initial velocities. However, differences in initial velocity between cultivars of smooth pea and pinto bean were not significant. Black bean, lentil and wrinkled pea starches showed a plateau at 93, 85 and 65% hydrolysis, respectively. The time taken for the appearance of the plateau was identical for the black bean cultivars, but was different for the lentil cultivars. Pinto bean and smooth pea cultivars showed no plateau. At the end of the assay period (120h), cultivars of each legume species were hydrolyzed to the same extent, and the extent of hydrolysis among the legume species followed the order: black bean > lentil > smooth pea > pinto bean > wrinkled pea. Scanning electron micrographs showed that starches were slightly eroded during the initial hydrolysis stage (<20% hydrolysis), but the integrity of most of the granules was well maintained. However, roughened surfaces and disc like depressions were obvious for all starches, except for lentil starch. No morphological differences were observed between cultivars from the same species for both native and hydrolyzed starches. The X-ray diffraction pattern and the 'B' polymorphic content of all starches remained unchanged upon hydrolysis. However, the relative crystallinity increased in wrinkled pea, but remained unchanged in the other starches. On hydrolysis, the apparent amylose content decreased in all starches. The extent of this decrease was most pronounced in wrinkled pea. In all starches, the enthalpy of gelatinization decreased, and the gelatinization transition temperatures increased slightly, on hydrolysis.

This study demonstrated that differences in the composition and physicochemical properties of starch between cultivars from the same species were marginal. The rate and extent of hydrolysis were influenced mainly by structural organization and interactions of the starch chains within the native granule, as well as by the extent of association between hydrolyzed amylose chains.

Keywords: Legume starches; physicochemical properties; α-amylase hydrolysis.

Chapter 1 Introduction

1.1 Legumes – General information

Legumes are dicotyledonous seeds of leguminous plants which belong to the Leguminosae family (Hoover and Sosulski, 1991). Leguminosae, containing about 650 genera and 18,000 species, is the third largest family of all flowering plants, after the Compositae and the Orchidaceae. Leguminosae comprises three sub-families: Caesalpinioideae, Mimosoideae and Papilionoideae. Papilionoideae is the largest and comprises 32 tribes compared with 5 in the Caesalpinioideae and 5 also in the Mimosoideae (Smartt, 1990; Sprent, 2001). Legumes are cultivated throughout the world and play an important role in the diets of many people.

1.1.1 Classification and production

In accordance with present Food and Agriculture Organization (FAO) practice, the word legume is used for all leguminous plants. For those containing only small amounts of fat, such as French beans, lima beans, etc., the term "pulse" is used and for those containing a high proportion of fat, such as soybeans and peanuts, the term "leguminous oilseed" is used. Although there are many legume species, only about 20 are commonly grown in different continents of the world and used for human consumption (Table 1-1). Of the various food legumes, soybean, peanut, dry bean, pea, broad bean, chickpea, and lentil are the major ones cultivated. Others are grown only in some countries, depending on the climatic conditions needed to support growth and the food habits of the consumers (Salunkhe and Kadam, 1989).

Table 1-1

Most common food legumes grown in the world (Salunkhe and Kadam, 1989)

Scientific name	Common name
Arachis hypogaea L.	Groundnut, peanut
Cajanus cajan (L.) Millsp.	Pigeonpea, red gram, Congo pea, Arhar, Tur, Gongo pea
Cicer arietinum L.	Chickpea, Bengal gram, garbanzo gram
Glycine max (L.) Merr.	Soybean, soya
Lablab purpureus (L.) Sweet	Hyacinth bean, Egyptian bean, Val.
Lathyrus sativus L.	Khesari, chickling vetch, grasspea
Lens culinaris Medik.	Lentil, Masur
Lupinus albus L.	White lupine
Lupinus angustifolius L.	Blue lupine, New Zealand blue lupine
Lupinus luteus L.	European yellow lupine
Macrotyloma uniflorum (Lam.) Verdc.	Horse gram, Madras gram, Kulthi
Phaseolus lunatus L.	Lima bean, butter bean
Phaseolus vulgaris L.	Bean, common bean, French bean, field bean, haricot bean, pinto bean, navy bean, dry bean
Pisum sativum L.	Common or garden pea, dry pea
Psophocarpus tetragonolobus (L.)	Winged bean, Goa bean, four-angled bean, Manila bean, princess pea
Vicia faba L.	Broad bean, faba bean, horse bean
Vigna aconitifolia (Jacq.) Marechal	Moth bean, mat bean
Vigna mungo (L.) Hopper	Urd, black gram
Vigna radiata (L.) Wilczek	Green gram, golden gram, mung bean
Vigna umbellata (Thumb.)	Rice bean, mambi bean
Vigna unguiculta (L.) Walp. ssp. *unguiculata*	Cowpea, black-eyed pea, crowder pea
Voandzeia subterranea (L.) Thouars	Bambarra groundnut

The total pulse production of the world in 2002 was 55,164,796 tons (Table 1-2). Canada and the EU are the main pea-producing countries and the Indian subcontinent and South America are the main regions producing dry beans (*Phaseolus* ssp. and *Vigna* spp.). The main region consuming pulses is the Indian subcontinent. In 1999, the food supply of pulses in Asia and India represented 56 and 36% of the world food supply of pulses, respectively. Africa and the Americas used 18 and 20% of the world supply, respectively (Schneider, 2002).

In Canada, pulses are mainly cultivated in Saskatchewan, Manitoba and Ontario. There has been a tremendous increase in pulse production during the past decade (Table 1-3). Canada is the world's largest exporter of dry pea and lentils and in 2000-2001, Canada also became the largest exporter of chickpeas. It also holds a significant share of the world's export of dry bean (Agriculture and Agri-Food Canada, 2003b).

1.1.2 Composition of legume seeds

The composition of legumes is governed by the cultivar, geographic location and growth condition. Simplified compositions of some legumes are summarized in Table 1-4; protein, carbohydrate and lipid are the major components. Generally, the composition of legumes includes approximately 15-45% protein, 24-68% carbohydrate and 0.8-49.7% lipid. Starch is the most abundant carbohydrate in legume seeds (22 – 45%). Some legumes such as peanuts, soy beans and chickpeas, are particularly high in their lipid and protein content, and contain about 45, 20 and 5% lipid, respectively, whereas soy beans, in addition, contain the highest amount of crude protein (~45%).

Table 1-2
World pulse production (Food and Agriculture Organization, 2003)

Continent	Production (MT)[1]
Africa	9,208,194
Asia	25,624,163
Europe	8,343,476
North & Central America	6,642,455
Oceania	1,393,020
South America	3,953,488
World Total	55,164,796

1. Million metric tons

Table 1 - 3
Canada pulse production (Agriculture and Agri-Food Canada, 2003a)

Pulse	Year	Production (KT)
Dry Pea	1991 - 1992	410
	1996 - 1997	1,169
	2003 - 2004f[1]	2,254
Lentil	1991 - 1992	343
	1996 - 1997	403
	2003 - 2004f[1]	550
Dry Beans	1991 - 1992	136
	1996 - 1997	133
	2003 - 2004f[1]	270
Chick pea	1991 - 1992	NA[2]
	1996 - 1997	4
	2003 - 2004f[1]	60

1. Forecast
2. Not available

1.1.3 Utilization and potential

Legumes are consumed as food in many parts of the world. Despite their relatively unfavorable protein quality (Gupta, 1983; Augustin and Klein, 1989; Friedman, 1996; Savage and Deo, 1989), they are regarded as an important source of protein in many developing countries. Extensive studies have been carried out to explore their functional properties and their uses as flours, protein isolates and concentrates (Sosulski *et al.*, 1976; Sosulski and Youngs, 1979; Vose, 1980; Sumner *et al.*, 1981; Sahasrabudhe *et al.*, 1981).

Legumes are also used as feed for animals. Pea is used extensively as a feed ingredient for cattle, swine, poultry and fish in Canada and the EU (Agriculture and Agri-Food Canada, 2003b; Waldroup and Smith, 1989).

Although starch is the most abundant carbohydrate in legume seeds, unlike other starches, such as wheat, corn, potato, and rice whose structure and functional properties have been studied extensively and have wide applications in industry and food products, legume starches have not been subjected to intensive research and neither have they been used widely by the food industry. Some researchers (Hoover and Sosulski, 1985a; Tjahjadi and Breene, 1984) have ascribed this to their lack of availability and high retrogradation rates. It was recently reported that the high retrogradation rates of legume starches could be reduced by chemical modification (Hoover and Sosulski, 1985b; Hoover *et al.,* 1988b) to levels that approach those of modified waxy maize starch. This, combined with their high thermal stability, should render legume starches advantageous for use in the food industry (Hoover and Sosulski, 1991).

Attention to legumes as a healthy food has been increasing recently. Firstly, legumes contain a relatively high fiber content (nondigestible food components) compared to other foods (Chen and Anderson, 1981), and this may be physiologically beneficial. Fermentation of the non-digestible food components (mainly dietary fiber and oligosaccharides) by anaerobic

Table 1-4
Composition of legume seeds (%)

Legumes	Protein		Fat		Total Carbohydrate		Starch
Groundnuts, peanuts	22.7	23.5 - 33.5	44.5	49.7	25.5	—	—
Pigeon peas, red gram	19.8	18.8 - 28.5	1.3	2.19	65.2	57.3 - 58.7	40.4 - 48.2
Chickpeas	19.5	14.9 - 29.6	5.7	4.99	61.7	—	—
Soybeans	34.3	33.2 - 45.2	18.7	21.3	31.6	25.4 - 33.5	0.2 - 0.9
Lentils	24.7	20.4 - 30.5	1.0	1.17	61.2	59.7	34.7 - 52.8
Lima beans	21.1	—	1.05	1.41	63.6	—	—
Black beans	21.8	21.2 - 31.3	1.4	1.64	63.5	56.5 - 63.7	32.2 - 47.9
Great Northern beans	22.0	—	1.2	3.0	63.4	61.2 - 61.5	44.0
Kidney beans	21.5	—	1.3	1.9	62.7	—	—
Navy beans	22.0	—	1.5	—	63.2	58.4	27.0 - 52.7
Pink beans	21.6	—	1.2	—	63.6	—	42.3
Pinto beans	21.4	—	1.2	1.85	64.1	58.4	51.0 - 56.5
Mung beans	23.6	—	1.4	—	61.6	53.3 - 61.2	37.0 - 53.6
Cowpea, black-eyed peas	22.0	20.9 - 34.6	1.3	1.5 - 2.05	63.4	56.0 - 68.0	31.5 - 48.0
References	a	b	a	b	a	b	b

a. Augustin and Klein (1989)
b. Kadam *et al.* (1989)

bacteria in the intestine gives rise to gas formation and to the formation of lactic acid and volatile fatty acids (VFA). These acids are reported to promote rapid intestinal transit of faeces and a more bulky, softer stool (Hellendoorn, 1978, 1979). Lack of fiber in the western diet is believed to result in constipation, and to be a main factor in the appearance of diverticular and colon-related diseases. Hellendoorn (1969, 1973, 1976, 1978, 1979) suggested that the ingestion of appreciable amounts of beans along with other foods eases or relieves constipation, and reduce the incidence of other colon related diseases.

Secondly, legumes are fairly resistant to attack by hydrolytic enzymes (Hoover and Sosulski, 1985a; Hoover and Sosulski, 1991; Hoover and Zhou, 2003; Dreher *et al.*, 1984) and contain high amounts of resistant starch (RS) (Juliano, 1999). As a consequence, legumes exhibit a lower digestion rates and slower release of glucose into the blood stream, resulting in reduced glycemic and insulinemic postprandial responses compared with other common foods such as cereal grains or potatoes (Jenkins *et al.*, 1982, 1988; Tovar *et al.*, 1992b; Bornet *et al.*, 1997). This is quite attractive to nutritionists, since it is helpful in the dietary control of diabetes as well as arterial disease. Several researchers have studied the digestibility and RS formation of raw and processed legumes (Hoover and Sosulski, 1985a; Björck *et al.,* 1994; Jenkins *et al.,*1982; Tovar *et al.,* 1992a; Tovar and Melito, 1996; Velasco *et al.,* 1997; Bravo *et al.,* 1998; Skrabanja *et al.,* 1999; Mahadevamma *et al.,* 2003).

In addition, legumes also show potential in the prevention of cardiovascular disease (Anderson and Major, 2002), cancers (Mathers, 2002), as well as lowering serum cholesterol concentrations (Geil and Anderson, 1994). Once known as poor man's meat, legumes are now facing a revival and are recommended for frequent consumption (Leterme, 2002).

1.2 Objectives of this research

The literature is replete with information on the susceptibility of cereal starches (A type crystallinity) towards hydrolysis by α-amylase. However, there is a dearth of information on the susceptibility of legume starches (C type crystallinity) towards hydrolysis by α-amylase. Legume starches differ widely in their amylose content, crystallinity, B-polymorphic content and magnitude of starch chain associations within the granule interior. They make better substrates than cereal and tuber starches for gaining a deeper insight into the structural factors that influence α-amylolysis due to the following reasons: 1) absence of pores on the granule surface (Hoover and Sosulski, 1985a); 2) absence of phosphate groups (Hoover and Sosulski, 1991); 3) presence of only trace quantities of bound lipids (Hoover and Sosulski, 1991); and 4) uniformity in granule size (Hoover and Sosulski, 1991). Thus, a comparative study of the susceptibility of legume starches (belonging to both the same and different biotypes) towards α-amylase may lead to the identification of structural factors that limit α-amylolysis.

Objectives of this research are:

1) To determine the composition and physicochemical properties of black bean (*Black Jack, CDC Nighthawk*), pinto bean (*Othello, Sierra*), smooth pea (*CDC Sonata, CDC Mozart*), lentil (*CDC Redwing, CDC Robin*) and wrinkled pea starches;
2) To study the susceptibility of the above starches towards porcine pancreatic α-amylase;
3) To determine changes in granular morphology, apparent amylose content, thermal properties, relative crystallinity and 'B' polymorphic content of starches during the time course of hydrolysis.

Chapter 2 Literature Review

2.1 Starch – general information

Starch is the second largest biomass, next to cellulose, produced on earth and is the major form of carbohydrate storage in green plants. Starch is abundant in many major agricultural crops and its content (dry basis) ranges from 40 to 90%, 65 to 85%, and 30 to 70% in cereals, roots and tubers, and pulses (legumes), respectively (Guilbot and Mercier, 1985). Most of the starches utilized world-wide come from a relatively small number of crops, the most important being maize, potato, wheat, and tapioca with smaller amounts from rice, sorghum, sweet potato, arrowroot, sago, and mung bean (Wang *et al.*, 1998). Legume starches are not utilized widely in the food industry due to their poor functional properties (Sosulski *et al.*, 1997; Ratnayake *et al.*, 2002).

Chemically, starch consists of two types of macromolecule, amylose and amylopectin. Amylose is essentially a linear molecule (Buléon *et al.*, 1998a) with a molecular weight of 5×10^5 Da to 10^6 Da and is composed of anhydroglucose units connected through α - 1,4 linkages. Amylopectin has a molecular weight of several millions and is a much branched polymer formed by anhydroglucose units mainly linked by α - 1,4 bonds, but additionally with 2 – 6% α - 1,6 linked branches (Hizukuri and Takagi, 1984; Takeda *et al.*, 1984, 1986; Buléon *et al.*, 1998a). "Normal" starches from most species contain about 25% amylose and 75% amylopectin (Eliasson and Gudmundsson, 1996). However, some mutant genotypes of maize (*Zea mays*), barley (*Hordeum vulgare*), and rice (*Oryza sativa*) contain as much as 70% amylose whereas other genotypes, called waxy, contain less than 1% amylose (maize, barley, rice, sorghum) (Buléon *et al.*, 1998a).

2.2 Starch production and utilization

The most common sources of starch are corn, potato, wheat, tapioca and rice. Maize (corn) is dominant in nearly all regions of the world, and wheat features only in Western Europe, and marginally North America and in Australia, while potato plays a role mainly in Europe, and tapioca essentially in Asia (Gordon, 1999). Legume starches have not been widely used due to their lack of availability and high retrogradation rates.

The world starch production was about 48.5 million tons in 2000 (Table 2-1), including not only native and modified starches, but also the large volume of starch that is converted into syrups for direct use as glucose and isoglucose, and as substrates in the form of very high dextrose syrups (known as starch hydrolysates) for fermentation into organic chemicals, including ethanol. Maize is the main raw material in all three regions listed in the table, supplying over 80% of the global starch production, along with 8, 5 and 5% from wheat, potato and other materials, respectively. Within the global industry, the US is the largest starch producer, with 51% of world production. The EU contributes more than 17% of world output, second only to the US.

Fig. 2-1 provides an overview of the wide range of products obtained from starch. LMC (2002) classified all starch products into four main categories: native and modified starches, ethanol, glucose and other syrup-based starch products. Products from these four categories accounted for 15, 40, 32 and 13%, respectively of the entire US starch output in 2002.

Starch, as a renewable and biodegradable resource, is abundant, environmentally friendly, cost competitive, and versatile. The variations in starch source, composition and structure, and the diversities in properties, make starch suitable for various applications contributing to

Table 2-1
Starch production by raw material in the EU, US and other countries, in 2000 (million metric tons)

	Maize	Potatoes	Wheat	Other	**Total**
EU	3.9	1.8	2.8	0	**8.4**
US	24.6	0.0	0.3	0	**24.9**
Other countries	10.9	0.8	1.1	2.5	**15.2**
World	39.4	2.6	4.1	2.5	**48.5**

Source: LMC International Ltd, 2002

Figure 2-1 Products derived from starch

Modified from Röper (2002)

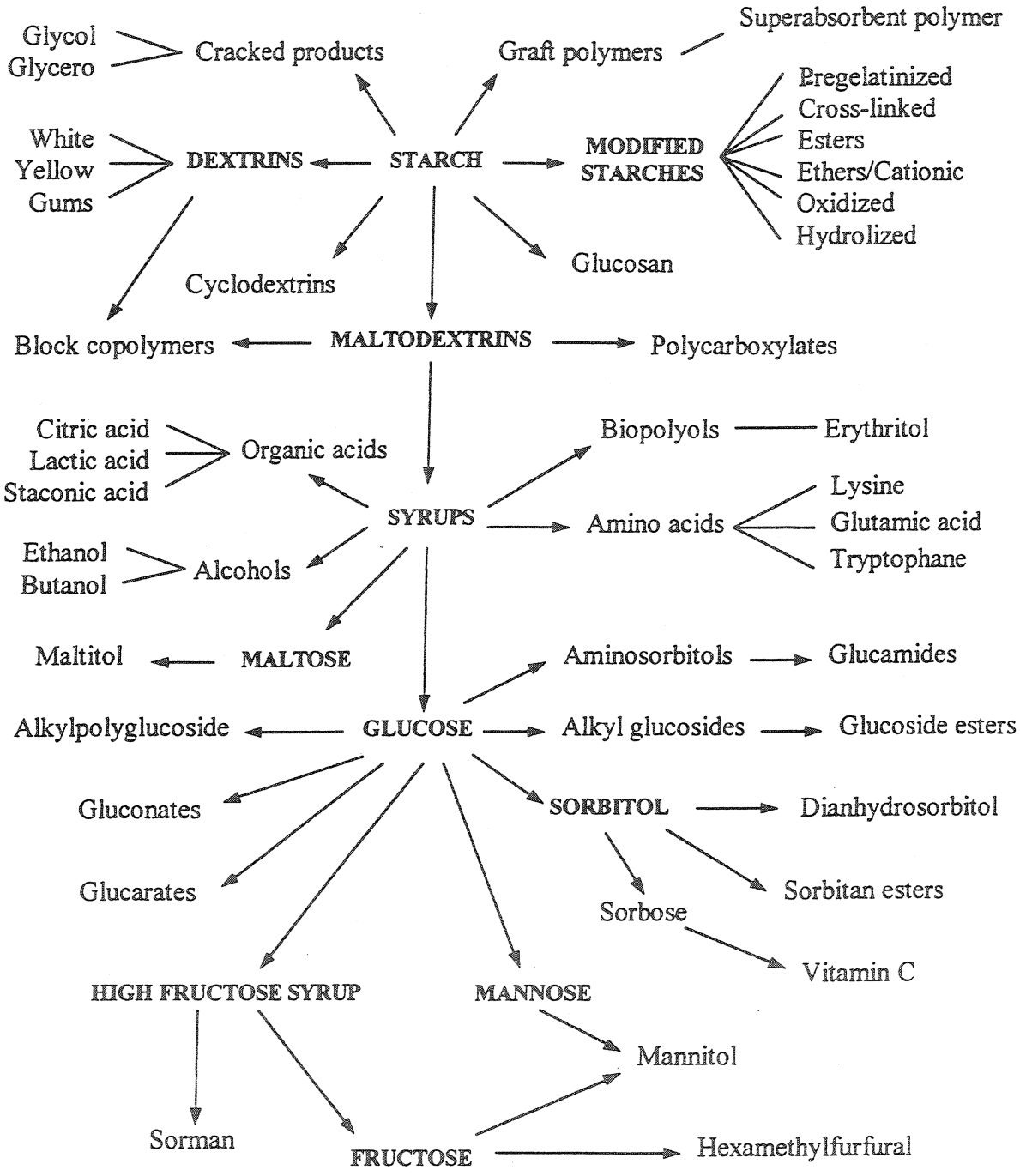

Glycol
Glycero
Cracked products
Graft polymers
Superabsorbent polymer
Pregelatinized
Cross-linked
Esters
Ethers/Cationic
Oxidized
Hydrolized
White
Yellow
Gums
DEXTRINS
STARCH
MODIFIED STARCHES
Cyclodextrins
Glucosan
Block copolymers
MALTODEXTRINS
Polycarboxylates
Citric acid
Lactic acid
Staconic acid
Organic acids
Biopolyols
Erythritol
SYRUPS
Amino acids
Lysine
Glutamic acid
Tryptophane
Ethanol
Butanol
Alcohols
Maltitol
MALTOSE
Aminosorbitols
Glucamides
Alkylpolyglucoside
GLUCOSE
Alkyl glucosides
Glucoside esters
Gluconates
SORBITOL
Dianhydrosorbitol
Glucarates
Sorbitan esters
Sorbose
Vitamin C
HIGH FRUCTOSE SYRUP
MANNOSE
Mannitol
Sorman
FRUCTOSE
Hexamethylfurfural

Table 2-2
Application of starch and starch derivatives in food and non-food industries.

Industry	Uses	Type used
Food	Thickener for pie filling, puddings stabilizer for salad dressings, frozen foods binder for meat and pet foods moisture retainer for bakery and meat fat replacer for desserts, spreads, sauces adhesive for food packing and meat gluing glaze for cakes, donuts, fruits and nuts	Native starches, modified starches, maltodextrins, high fructose syrups
Beverage	Soft drinks, beer, alcohol, instant coffee	Sweeteners
Confectionery	Ice cream, candy, gums, marshmallows, canning, marmalade and jams	Starch, maltodextrins, maltose syrups
Adhesive	Case sealing, laminating, tube winding, corrugated board	Starch and dextrins
Paper & Cardboard	Wet end additives, spraying, surface sizing, coating	Native, cationic, and hydroxyethyl starches
Textile	Sizing, finishing, printing, fire resistances	Starch and modified
Cosmetic	Emulsifiers, humectants, face powders	Starch, sorbitan esters
Detergent	Surfactants, builders, bleach activators	Sucrose derivatives
Pharmaceuticals	Diluents, binders, drug delivery, encapsulation	Starch, malto- and cyclodextrins, glucose syrups, polyols
Plastics	Biodegradable filler	Starch
Biochemistry	Organic acids, amino acids, biopolymers, polyols, enzymes, alcohols, antibiotics	Starch hydrolysates
Other	Ceramics, coal, water treatment, gypsum and mineral fiber, oil drilling, concrete	Starch and modified starches

Source: Ellis et al. (1998); Guzmán-Maldonado and Paredes-López (1995); Lillford and Morrison (1997).

different functions. Native starches have diverse properties, which meet different application requirements. However, physical and chemical modifications greatly improve the properties of native starches and extend the range of starch applications in food, paper and board, textiles, and pharmaceuticals. Also, numerous starch derivatives produced by enzymatic technology, including dextrins and various DE syrups, have been used for production of organic acids, solvents, alcohols, amino acids, biopolymers and other products. A wide range of applications for starch and starch derivatives in various industries are summarized in Table 2-2.

2.3 Starch composition and structure

2.3.1 Composition

Starch usually contains 10 – 15% moisture, 85 – 90% polysaccharides (amylose and amylopectin), and minor non-polysaccharide components (protein, lipid and minerals) (Tester, 1997). In legume starches (Table 2–3), protein, ash and lipid contents range from 0.10 to 1.12%, 0.03 to 0.81%, and 0.01 to 0.87%, respectively.

2.3.1.1 Major components

Amylose and amylopectin are the principal components of starch and their content varies depending on starch source, species and cultivar (Galliard and Bowler, 1987). Normally, non-mutant starches from most species contain about 25% amylose and 75% amylopectin (Eliasson and Gudmundsson, 1996). The amylose content of legume starches (Table 2–3) ranges from 19.5 to 75.4%.

Table 2 – 3

Composition of legume starches[1,2,3]

Starch source	Yield of pure starch (%)	Protein (%)	Lipid (%)	Ash (%)	Amylose (%)	Iodine affinity (%)
Kidney bean	25	0.13 - 0.30	0.18	0.18	34.4 - 35.0	7.02 - 8.04
Northern bean	18 - 31	0.35 - 0.97	0.20 - 0.46	—	31.6	—
Navy bean	21 - 40	0.13 - 0.34	0.09 - 0.60	0.06 - 0.14	36	6.58 - 7.20
Black bean	32	0.55 - 1.12	0.15	0.11	35.1 - 37.3	6.82 - 7.20
Mung bean	32 - 43	0.12 - 0.28	0.17 - 0.50	0.18 - 0.27	19.5 - 40	5.95 - 6.98
Pinto bean	27 - 38	0.37 - 0.52	0.16 - 0.51	0.05 - 0.09	25.8 - 30.2	—
Adzuki bean	21.5	0.1 - 0.27	0.03 - 0.06	0.07 - 0.19	21.2 - 34.9	6.98
Moth bean	33.5	0.58	0.87	0.62	26.4	5.81
Faba bean	39.9	0.49 - 0.52	0 - 0.08	0.06	31.3 - 42.1	6.03 - 5.61
Horse bean	37	0.16 - 0.90	0.06	0.81	24 - 32	4.50 - 6.28
Lima bean	23 - 30	0.22 - 0.44	0.1	0.07 - 0.13	—	6.56 - 6.60
Red bean	46.3	0.13	0.01	0.05	35.7	4.83
Lablab bean	—	0.21	0.2	0.03	30	6.05
Smooth pea	40	0.52 - 0.70	0.01 - 0.1	0.07	32.5 - 33	6.98
Wrinkled pea	18 - 22	0.34 - 0.46	0.01 - 0.19	0.08	62.8 - 75.4	12.80 - 15.18
Black gram	45	—	—	—	26.65	—
Chick pea	40	0.70 - 0.94	0.06	0.07	30.4 - 32.2	6.08
Cow pea	37	0.12 - 0.50	0.21 - 0.33	0.06	33	6.6
Horse gram	28	0.05	—	0.05	34.3	—
Lentil	25 - 42	0.17 - 0.53	0.05 - 0.23	0.13	29 - 45.5	6.97 - 9.09

1. Source: Hoover and Sosulski (1991)
2. For tuber and root starches, the nitrogen, lipid, phosphorous, and amylose content are in the range 0.006 - 0.49%, 0.006 - 3.96%, 0.003 - 0.08% and 10 - 38%, respectively (Hoover, 2001; Narayana Moorthy, 2002).
3. For barley starches, the protein, lipid, phosphorous, and amylose content are in the range 0.25 - 0.56%, 0.16 - 1.17%, 0.022 - 0.068%, and 1.8 - 47.9%, respectively (Morrison *et al.*, 1986; McDonald and Stark, 1988; Ellis *et al.*, 1998; Kasemsuwan and Jane, 1996; Lim *et al.*, 1994; Song and Jane, 2000).

2.3.1.2 Minor components

In addition to amylose and amylopectin, starch contains small amounts of proteins and lipids, as well as trace amounts of minerals. Lipid and protein may occur either on the surface of or inside, the starch granule (Lillfford and Morrison, 1997). Although these components occur in small amounts, their presence greatly influences the properties of starch granules such as digestibility, swelling ability, solubility, retrogradation, and granule integrity (Han and Hamaker, 2002; Appelqvist and Debet, 1997; Galliard and Bowler, 1987).

2.3.1.2.1 Lipids

Lipids in cereal, tuber, root and legume starch granules have been found to occur both on the surface of and inside the granule (Morrison, 1981), The surface lipids are mainly triacylglycerides, followed by free fatty acids, glycolipids and phospholipids (Morrison, 1981; Galliard and Bowler, 1987; Vasanthan and Hoover, 1992). The internal lipids mostly are monoacylglycerides, with the major components being lysophospholipids and free fatty acids (Hargin and Morrison, 1980; Morrison, 1981; Vasanthan and Hoover, 1992). Total starch lipids (surface plus internal) have been found to be generally in the range of 0.7–1.2% in cereals (Morrison and Milligan, 1982; Takahashi and Seib, 1988; Vasanthan and Hoover, 1992), 0.01–0.87% in legumes (Table 2–3) and 0.08–0.19% in tubers and roots (Emiola and Delarosa, 1981; Goshima *et al.*, 1985; Vasanthan and Hoover, 1992).

Starch lipids may be present in a free state as well as bound to other starch components, linked via ionic or hydrogen bonding to hydroxyl groups of the starch components or in the form of an amylose-lipid complex in which the ligand resides within the central hydrophobic center of the helix (Morrison, 1981). Free lipids are easily extracted by using chloroform-methanol (2:1, v/v) at ambient temperature, whereas bound lipids need many hours of extraction with hot aqueous solvent [n-propanol

– water (3:1, v/v)] or acid hydrolysis to completely disrupt the starch granule before the lipids are released (Morrison, 1981; Goshima *et al.*, 1985; Hoover *et al.*, 1988a; Vasanthan and Hoover, 1992).

2.3.1.2.2 Proteins

Proteins are also associated with starch granules and their amounts vary among and within species. Typical protein contents of wheat, maize and potato is 0.2–0.3, 0.35 and 0.06%, respectively (Skerritt *et al.*, 1990; Swinkels, 1985). In general, ten principal polypeptide bands are evident in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separations of starch granule-associated protein extracts. These bands correspond to proteins with molecular weights ranging from ~5 to 149 kDa. As a function of their molecular weights, the proteins have been classified into two groups: surface proteins with low molecular weights (~5, 8, 15, 19 and 30 kDa) and internal proteins with higher molecular weights (~60,77, 86, 95 and 149 kDa) (Baldwin, 2001).

2.3.1.2.3 Minerals and phosphorous

Starches contain trace amounts of minerals such as Ca^{2+}, K^{+}, Mg^{2+} and Zn^{2+} as well as bound phosphorous. Most of the phosphorous in cereal starches is present in the form of lysophospholipid (0.02–0.06%) (Morrison, 1995), whereas waxy starches have much less phosphorous (<0.01%), mainly in the form of starch phosphate monoesters. High–amylose corn starch (70% amylose) contains organic phosphorous (0.02%) as starch phosphate monoesters and phospholipids in a 1:4 ratio. Root and tuber starches are phospholipid-free, the phosphorous is mainly in the form of phosphate monoesters. Legume starches contain ~0.01% phosphorous, mainly in the form of phosphate monoesters which are exceptionally high in potato starch (~0.089%). Phosphate monoesters of all

starches are located more on the primary carbon (C-6) than on the secondary carbon (C-3) of the anhydrous glucose unit of amylopectin (Lim *et al.*, 1994; Kasemsuwan and Jane, 1996).

2.3.2 Ultrastructure

2.3.2.1 Overview of granule structure

The structural basis of starch granules is amylose and amylopectin. Amylose is essentially a linear molecule linked by α-(1,4)-D-glycopyranosyl units. Amylopectin is highly branched, formed from chains of α-D-glucopyranosyl residues linked together mainly by (1→4) linkages but with 5 – 6% of (1→6) bonds at the branch points (Buléon *et al.*, 1998a). Amylose and amylopectin chains further form crystalline and amorphous regions of the starch granule. Starch is biosynthesized as semi-crystalline granules with varying polymorphic types and degrees of crystallinity. Granule crystallinity has been mainly attributed to the amylopectin fraction.

The inner architecture of the native starch granule is characterized by growth rings (Yamaguchi *et al.*, 1979; Jenkins *et al.*, 1993; Donald *et al.*, 1997) that represent concentric crystalline shells or layers separated by amorphous regions. A model of the arrangement of the amorphous and crystalline regions is schematically shown in Fig. 2-2. Regions of amylopectin double helices fall within the crystalline lamellae, while the amylopectin branch points lie in the amorphous lamellae. The crystalline lamellae exist alternatively with the amorphous lamellae (Fig. 2-2B). The combined thickness of crystalline plus amorphous lamellae is 9 and 9.2nm for A-type and B-type starches, respectively (Jenkins *et al.*, 1993).

Gallant *et al.* (1997) recently revealed the different levels of structural organization within the starch granule. They proposed the presence of blocklets having dimensions of a few hundred nanometers within the growth rings. Helbert and Chanzy (1996) suggested that amylopectin is

composed of "superclusters" having sizes similar to that of the blocklets. Oostergetel and Van Bruggen (1993) proposed a three-dimensional helical structure for the lamellae organization in lintnerized potato starch, suggesting the occurrence of a "superhelical" organization. Based on these results, the amylopectin side chain clusters have been suggested to form the submacroscopic structure of the blocklets. The blocklets range in diameter from approximately 20 to 500nm depending on starch type (botanical source) and location in the granule. Starches resistant to enzymatic attack (potato and high-amylose starches) have been shown to consist of larger blocklets than less resistant starches (Gallant *et al.*, 1992). At the lowest level of granule organization, the starch granules contain alternating hard (crystalline) and soft (semicrystalline) shells of several hundred nanometer thickness. The hard shells are composed of larger blocklets (50 to 500nm) than the soft shells, where the blocklet size ranges between 20 and 50nm. The repeat of a hard and a soft shell has been regarded as a growth ring, which can be observed using light microscopy. The width of the shells becomes progressively thinner toward the exterior of the starch granules. A detailed schematic illustration of this blocklet structure is shown in Fig. 2-3.

2.3.2.2 Amylose

Amylose constitutes about 25% of the starch granule. Generally, it is a linear molecule linked by α-(1,4)-D-glycopyranosyl units (Fig. 2-4). However, a slight degree of branching [9 – 20 α(1→6) branch points per molecule] has been reported in amylose from various starch sources. The side chains range in chain length from 4 to over 100 (Hizukuri *et al.*, 1981; Takeda *et al.*, 1987a). The extent of branching has been shown to increase with the molecular size of amylose (Greenwood and Thomson, 1959). Evidence of the occurrence of branch points in amylose is its incomplete conversion into

Figure 2-2 Internal structure of a starch granule showing alternating amorphous and semi-crystalline growth rings

A) Stacks of semi-crystalline lamellae are separated by amorphous growth rings.

B) A magnified view of one such stack, showing that it is made up of alternating crystalline and amorphous lamellae.

C) The crystalline lamellae comprise regions of lined up double helices formed from amylopectin branches. The amorphous lamellae are where the amylopectin branch points sit.

Source: Donald *et al.* (1997), reproduced with permission.

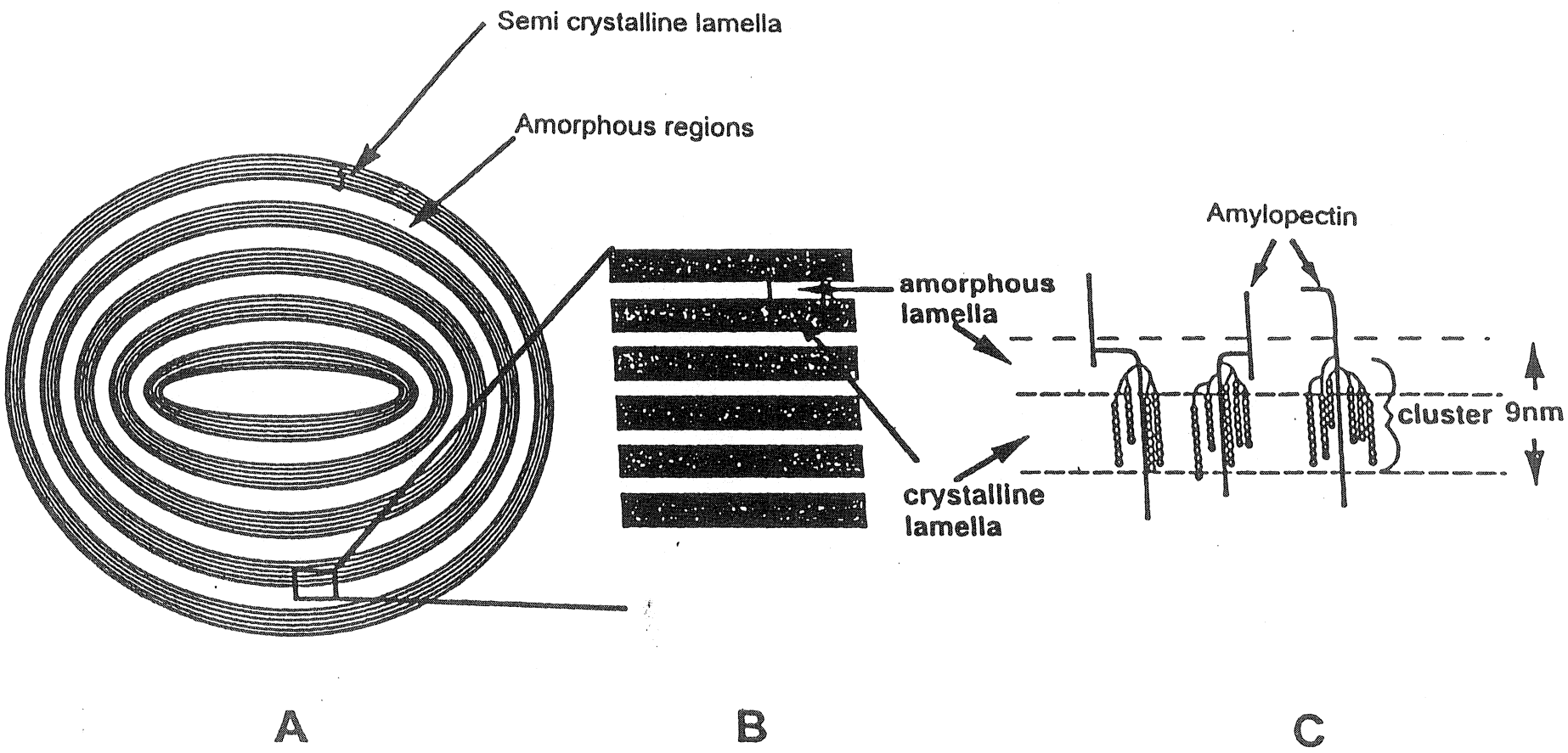

Semi crystalline lamella
Amorphous regions
amorphous lamella
crystalline lamella
Amylopectin
cluster
9nm
A
B
C

Figure 2-3 Blocklet model of starch granule structure

(a) The granule is composed of alternating crystalline (hard) and semi-crystalline (soft) shells (dark and light color, respectively). The shells are thinner towards the granule exterior (due to increasing surface area to be added to by constant growth rate) and the hilum is shown off centre.

(b) Blocklet structure is shown, in association with amorphous radial channels. Blocklet size is smaller in the semi-crystalline shells than in the crystalline shells.

(c) One blocklet is shown containing several amorphous and crystalline lamellae. The next diagram shows the magnified picture of amorphous and crystalline lamellae of amylopectin.

(d) Amylose-lipid (and protein) complexes feature in the organization of the amylopectin chains.

(e) The crystal structures of A and B type crystalline.

Source: Gallant *et al.* (1997), reproduced with permission.

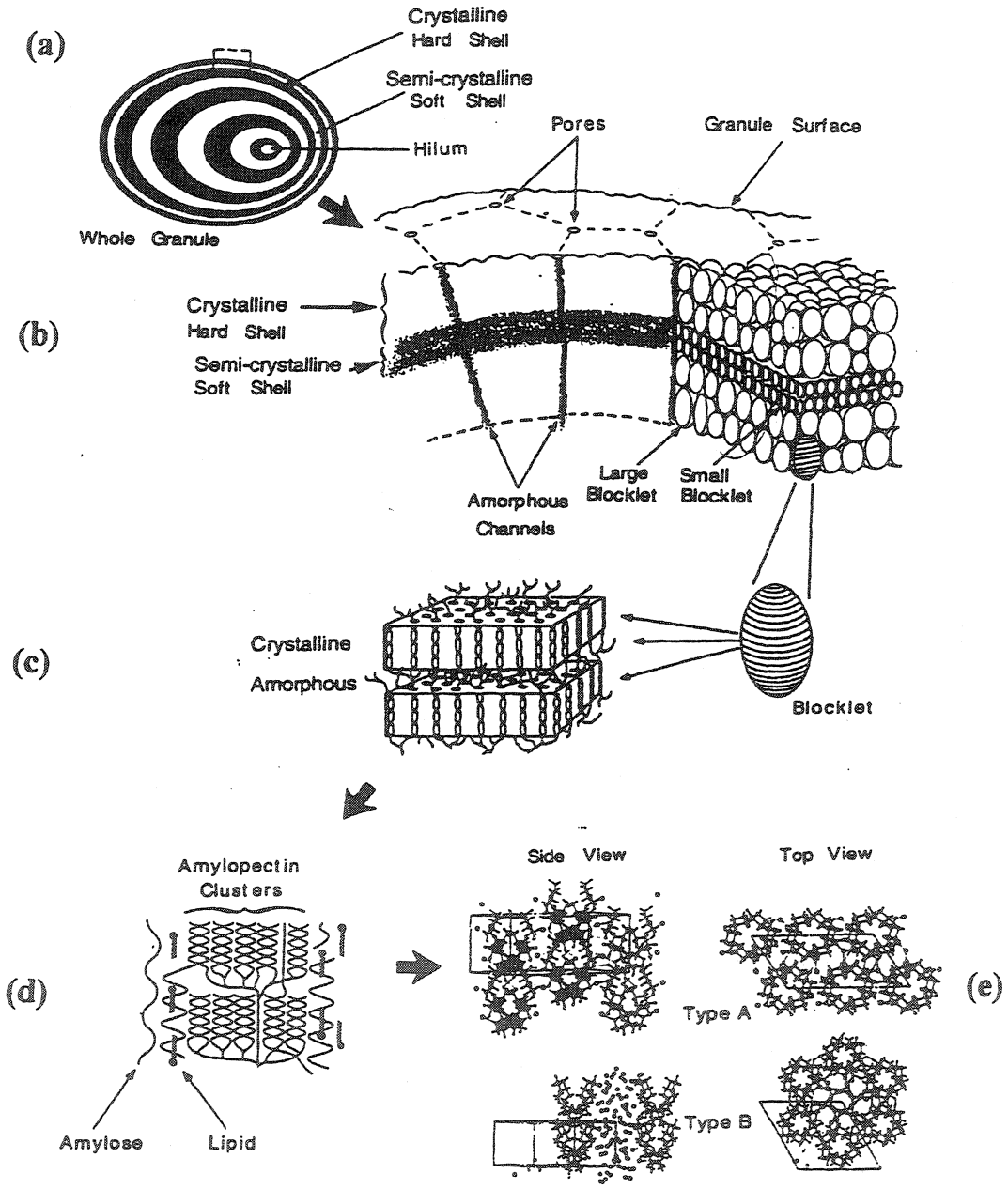
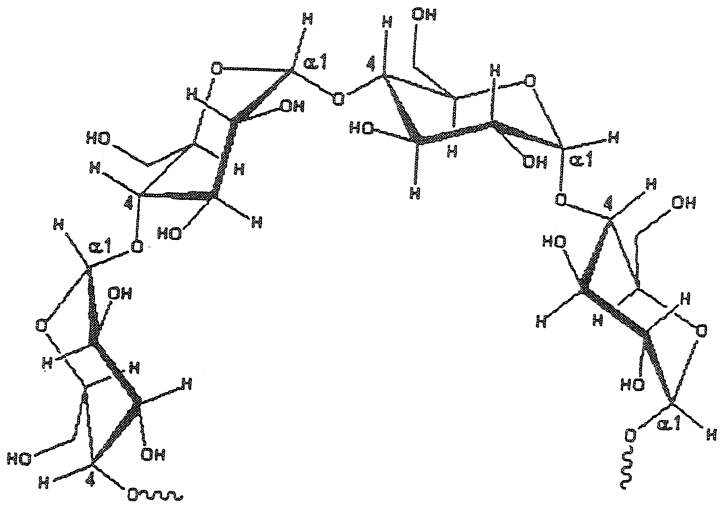

(a)
Crystalline
Hard Shell
Semi-crystalline
Soft Shell
Hilum
Whole Granule
Pores
Granule Surface
(b)
Crystalline
Hard Shell
Semi-crystalline
Soft Shell
Amorphous
Channels
Large
Blocklet
Small
Blocklet
(c)
Crystalline
Amorphous
Blocklet
(d)
Amylopectin
Clusters
Amylose
Lipid
Side View
Top View
Type A
Type B
(e)

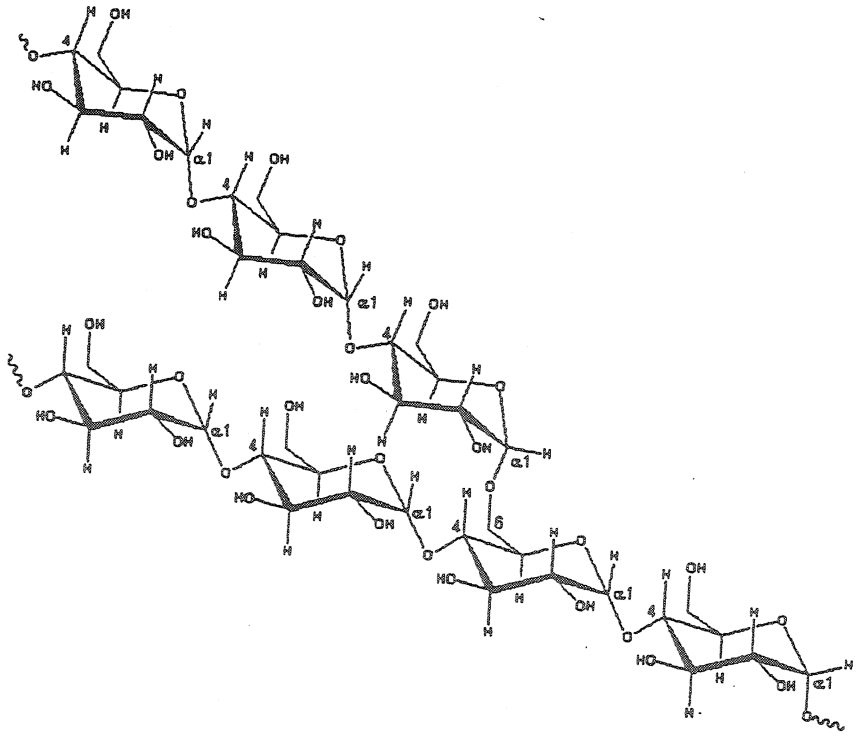
Figure 2–4 Representative partial structure of amylose (A) and amylopectin (B)

Source: LSBU (London South Bank University) (2003)

(A)



(B)



maltose by β-amylase; β-amylolysis has been shown to vary from 73 to 95% (Morrison and Karkalas, 1990). The molecular weight of amylose has been reported to vary between 10^5 and 10^6 Da (Morrison and Karkalas, 1990; Hizukuri *et al.*, 1989). Table 2–4 summarizes the general characteristics of amylose and amylopectin.

The physicochemical characteristics of some legume amyloses are presented in Table 2–5. The iodine binding capacity (IBC), limiting viscosity number (η), degree of polymerization (DP) and β-amylolysis limit for amylose from legume starches are in the range of 16–22, 136–280, 1000–1900, and 79–86.9%, respectively. The molecular weights determined only on selected legume amyloses range from 165,000 to 312,000 Da. The DP values of legume amyloses are lower than those of tuber and root starches, which are 3400–4100, 2110–4920, 2660 for sweet potato, potato and tapioca, respectively (Hizukuri, 1996).

2.3.2.2.1 Amylose inclusion complexes

Despite its slightly branched nature, in neutral solutions and other solvents such as dimethylsulfoxide (DMSO), formamide and aqueous alkali, amylose behaves as a random coil, assuming a helical conformation in the presence of a complexing agent (Banks and Greenwood, 1971, 1975). It is well known that amylose can form inclusion complexes with guest molecules such as iodine, alcohols and certain lipids. The crystalline structure of the amylose-ligand complex is commonly referred to as V-polymorph. Amylose, in the single helical (V) conformation, has six glucosyl residues per turn (with bulky ligands there are seven or eight), stabilized by hydrogen bonds between the hydroxyl groups of adjacent glucosyl residues, 2-OH — 3'-OH and 2-OH — 6'-OH interturn hydrogen bonds, and numerous intra- and intermolecular van der Waals contacts located on the outer surface of the helix (Banks and Greenwood, 1975; Rappenecker and Zugenmaier, 1981; Blanshard, 1987; Biliaderis, 1998). The helix cavity is

effectively a hydrophobic channel which provides a place to accommodate the guest molecules during complex formation.

2.3.2.2.1.1 Amylose – lipid complex

The formation of amylose–lipid complexes (ALC) can occur *in situ* with natural fatty acids and phospholipids during the biosynthesis of starch. Amylose-lipid complexes can also be formed during gelatinization of starch in the presence of naturally occurring lipids or added food emulsifiers such as monoacylglycerides, sodium or calcium stearoyl lactylate, and sorbitan monostearate. Fig. 2–5 is a schematic illustration of an ALC. The hydrophobic chain of the fatty acid or lipid lies inside the amylose helix and is stabilized by van der Waals contacts with the adjacent C (5) – hydrogen of amylose, but the polar ends of the lipids are not inside the helix cavity (Godet *et al.*, 1993). The ' V' – X-ray pattern of ALC'S is found in high-amylose starches, and in starches containing genes such as amylose extender and in dull or sugary starches (Zobel, 1988a). Proof that ALC'S do exist in native starch granules and that they are not artifacts formed during starch isolation was obtained by ^{13}C CP/MAS-NMR spectroscopy (Morrison *et al.*, 1993a, 1993b, 1993c).

2.3.2.2.1.2 Amylose-iodine complex

The reaction between amylose and iodine has been known for over a century. Rundle and Baldwin (1943) proposed that the iodine component of the complex is present in a unidimensional array within any amylose helix with six glucose residues per turn. Teitelbaum *et al.* (1978, 1980) studied the structure of the amylose-iodine complex using Raman and Mossbauer spectroscopy and postulated that the principal chromophore was pentaiodine anion (I^{5-}). The blue color formed by amylose-iodine complex has served as the principle for

Table 2 - 4
General characteristics of amylose and amylopectin

Property	Amylose	Amylopectin
Molecular structure	Essentially linear, α-1,4-glucosidic linkage	Branched, α-1,4 and α-1,6-glucosidic linkage
Degree of branching (%)	0.2 - 0.7	4.0 - 5.5
Degree of polymerization (DP)	700 - 5000	10^4 - 10^5
Molecular weight (Da)	10^5 - 10^6	10^7 - 10^9
Average chain length	100 - 550	18 - 25
Structural conformation	Partly complexed with lipid, amorphous	Double helix, partly crystalline
Iodine complex		
Iodine affinity (g/100g)	19- 20.5	0 - 1.2
λ_{max} (nm)	640 - 660	530 - 570
Blue value	1.2 - 1.6	0 - 0.2
Color	Blue	Purple
β-amylolysis limit (%)	70 - 95	55 - 60
Stability of dilute aqueous solutions	Unstable (retrogrades)	Stable
Gel texture	Stiff, thermally irreversible (<100°C)	Soft, thermally reversible (<100°C)
Film properties	Strong, coherent	Brittle
Reference	Biliaderis, 1991; Hizukuri, 1996	

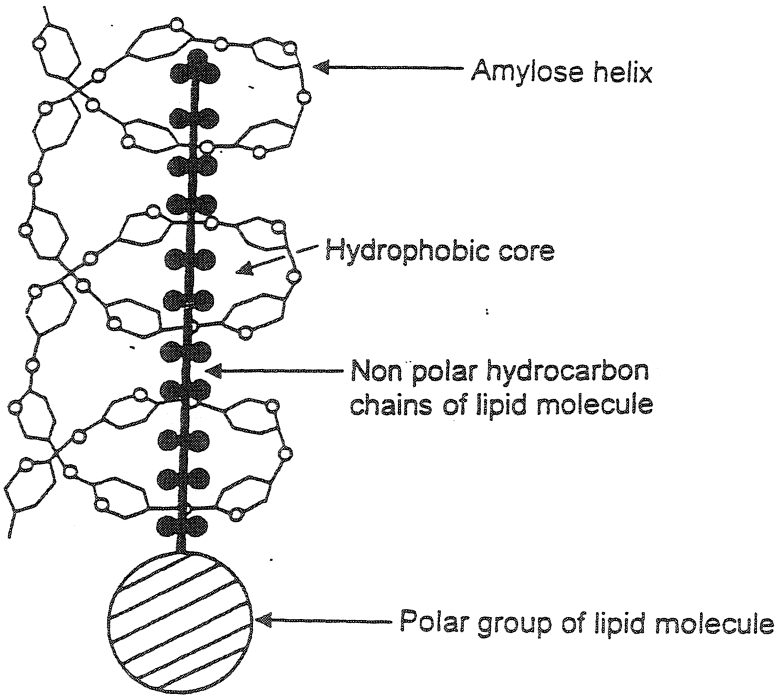
Table 2 - 5
Physicochemical characteristics of legume amyloses

Starch source	Iodine binding capacity	Limiting viscosity number (mL/g)	Degree of Polymerization	Molecular weight	β-Amylolysis
Kidney bean[a]	20	180	1300	—	85.9
Navy bean[a]	18.48	174	1300	165 000	86.2
Black bean[a]	22.01	—	—	—	
Mung bean[a]	19.43	251	1900	245 000	78.4
Pinto bean[a]	—	—	—	123 000	—
Adzuki bean[a]	16 - 19.49	220	1600	—	86.8
Adzuki bean[b]	19.4	—	1350	—	83
Faba bean[a]	19.61	188	1400	191 000	85.6
Horse bean[a]	17.1 - 19.2	240 - 280	1800	—	82
Smooth pea[a]	18.84 - 19.2	136 - 150	1000 - 1100	125 000	79 - 84.7
Pea[b]	19	—	820	—	90
Field pea[c]	—	—	1300 - 1350	—	—
Lentil[a]	19.62	188	1400	312 000	89.4

a. Hoover and Sosulski (1991)
b. Yoshimoto *et al.* (2001)
c. Ratnayake *et al.* (2001)

Figure 2 – 5 Schematic illustration of amylose-lipid complex

Source: Carlson *et al.* (1979), reproduced with permission


Amylose helix
Hydrophobic core
Non polar hydrocarbon chains of lipid molecule
Polar group of lipid molecule

colorimetric methods to determine amylose content. Amylose can bind some 20% of its weight of iodine (Biliaderis, 1998). However, the color of the complex may vary as a result of the chain length (DP) of amylose, which influences its binding ability with iodide anions. John *et al.* (1983) reported that the color of the complexes changed from brown (DP 21 – 24) to red (DP 25 – 29), red-violet (DP 30 – 38), blue-violet (DP 39 – 46), and finally blue (DP>47). When DP is lower than 20, no color was formed.

2.3.2.2.2 Location of amylose

The location of amylose in a starch granule is still in dispute. The possible locations are listed as follows: (1) amorphous growth ring; (2) amorphous lamellae of semi-crystalline growth ring (between crystalline lamellae); (3) interspersed or co-crystallized with amylopectin molecules (Jenkins and Donald, 1995; Jane *et al.*, 1992; Kasemsuwan and Jane, 1994); (4) radial channels and central cavities (Gallant *et al.*, 1997).

Amylose has been located in bundles between amylopectin clusters (Nikuni, 1978; Blanshard, 1986; Zobel, 1992). However, by using epichlorohydrin cross-linking, phosphorous-31nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography, other researchers (Jane *et al.*, 1992; Kasemsuwan and Jane, 1994) have shown that amylose molecules are randomly interspersed among amylopectin clusters in both the amorphous and crystalline regions instead of being in bundles. Reports about the distribution of amylose in starch granules are also contradictory. Increased values of blue value, iodine affinity, and amount of amylose fraction during granule development in maize (Inouchi *et al.*, 1984), wheat (Morrison and Gadan, 1987), barley (McDonald *et al.*, 1991), rice (Asaoka *et al.*, 1985), pea (Biliaderis, 1982a), and potato (Jane and Shen, 1993) starches imply a richer amylose region in the periphery region than in the center of the granules.

However, reverse amylose distribution in potato and maize hybrid *ae/wx* starch granules with reduced amylose content has also been reported (Schwatz, 1982; Yun and Matheson, 1992; Kuipers *et al.*, 1994; Tatge *et al.*, 1999). The independent localization of amylose and amylopectin in starch granules with varying amylose contents (0 – 70%) by enzyme-gold labeling (Atkin *et al.*, 1999) revealed that the location of amylose differed with different amylose contents. Amylose in low amylose content potato starch was mainly located in the amorphous growth rings alternating with semi-crystalline growth rings, whereas high amylose content (amylomaize) granules were shown to possess an amylopectin center surrounded by an amylose periphery encapsulated by an amylopectin surface.

Based on the fact that increasing amylose content has the effect of increasing the crystalline region size but reducing the electron density of small angle x-ray scattering (SAXS), Jenkins and Donald (1995) suggested that increased amylose acts to disrupt the packing of the amylopectin double helices within the crystalline lamellae. Two mechanisms were provided. Mechanism 1 (Fig. 2–6) involves amylose co-crystallizing with amylopectin. Mechanism 2 involves amylose chains oriented transverse to the lamella stack, penetrating the amorphous lamellae and introducing disorder. However, current evidence is insufficient to distinguish these two mechanisms.

2.3.2.3 Amylopectin

Amylopectin constitutes about 75% of most starches. It is a highly branched macromolecule formed through chains of α-D-glucopyranosyl residues linked together mainly by (1→ 4) linkages but with 5~6% of (1→ 6) bonds at the branch points (Buléon *et al.*, 1998a). Amylopectin is one of the largest polymers in the nature, with a M_r (weight average molecular weight) of the order $10^7 \sim 10^9$ (Aberle *et al.*, 1994). Some general characteristics of amylopectin are listed in Table 2–4. The average size of the repeating unit chains of amylopectin is in the range of 20-25 (Hizukuri, 1985).

Extensive studies have been carried out on amylopoectin structure in terms of molecular size, branch chain length (inner and outer) distribution, location of branch points, and crystallinity by various techniques, such as chemical and enzymatic analysis including chromatography, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), fluorophore-assisted carbohydrate electrophoresis (FACE) (Broberg *et al.*, 2000; Wang *et al.*, 2000; Morell *et al.*, 1998; Hizukuri, 1996; Morrison and Karkalas, 1990; Manners, 1989), optical and electron microscopy (Gallant *et al.*, 1997; French, 1984), nuclear magnetic resonance (NMR) spectroscopy (Gidley, 2001; Morgan *et al.*, 1995; Gidley and Bociek, 1985), and X-ray and neutron scattering (Donald *et al.*, 1997, 2001). A number of reviews on amylopectin structure have been published (Gidley, 2001; Tester *et al.*, 2001; Gallant, 1997; Oates, 1997; Hizukuri, 1996; Ball *et al.*, 1996; Gidley and Cooke, 1991; Imberty *et al.*, 1991; Manners, 1989; Zobel, 1988a, 1992; Sarko and Zugenmaier, 1980; Banks and Greenwood, 1975).

2.3.2.3.1 Structural model

It is now widely accepted that amylopectin is structured in a cluster model (Fig. 2–7). Three types of unit chains are present, referred to as A-, B-and C-chains. A-chains are unbranched and linked to B- chains through α–1,6–bonds at their reducing end-group. B-chains are linked to other B-chains or C- chain in the same manner, carrying either A- chains or B-chains. C-chains is the only chain with a reducing end-group and carrying numerous B-chains. A- chains form crystalline lamellae and their α-(1,6)- branch points are located in amorphous lamellae. Some B-chains are long enough to traverse through both the semi-crystalline growth ring and the inter-crystalline amorphous growth ring.

The ratio of A-chains to B-chains, which is also referred to as the degree of multiple branching, is an important parameter. Manner and Matheson (1981) have shown the A:B ratio to be 0.8

Figure 2 – 6 One possible mechanism to explain the disruption of amylopectin double helical packing by amylose.

(A) Amylopectin structure with no amylose present. Small crystalline lamella size.

(B) Co-crystallizing between amylose and amylopectin pulls a number of the amylopectin chains out of register. The crystalline lamella size increases.

Source: Jenkins and Donald (1995), reproduced with permission.

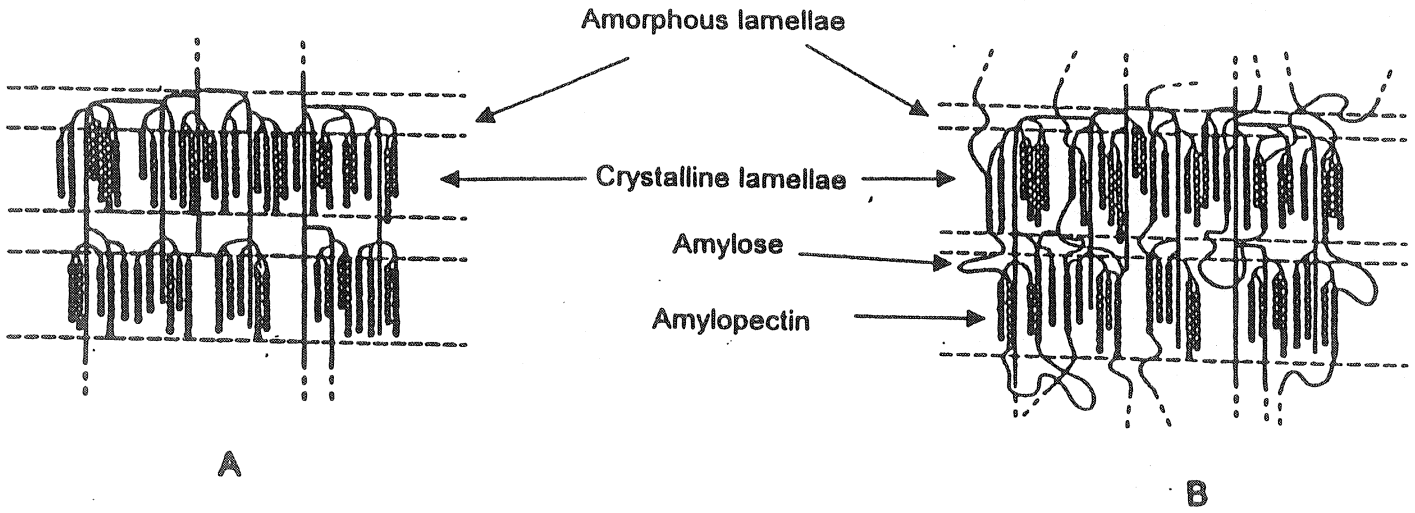
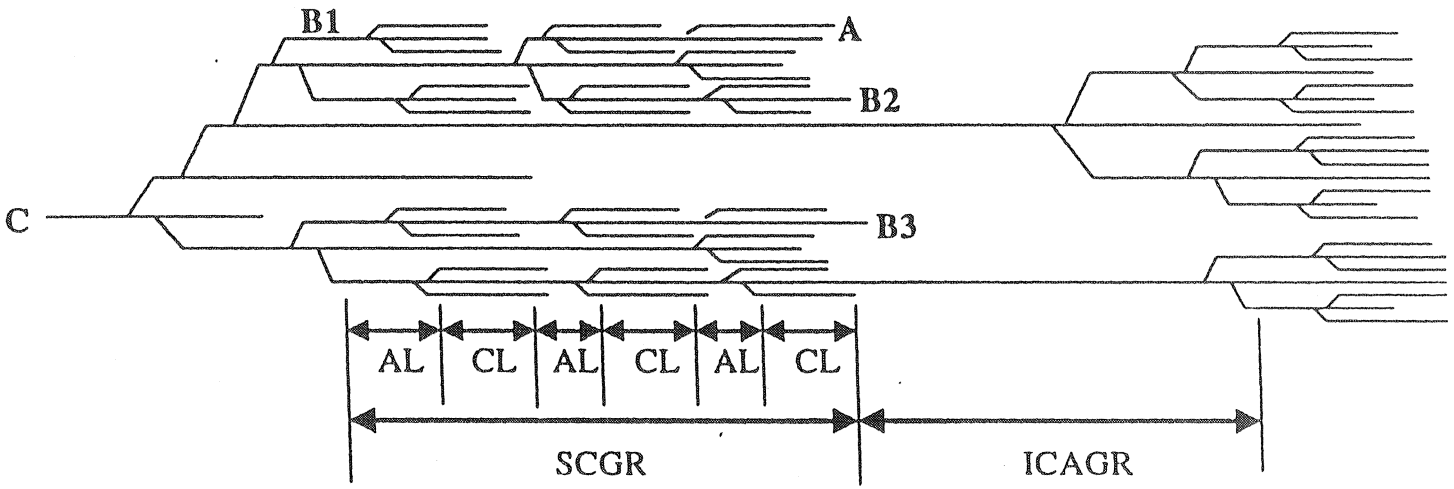

Amorphous lamellae
Crystalline lamellae
Amylose
Amylopectin
A
B

Figure 2 – 7 Cluster structure of amylopectin showing growth rings and areas for amorphous and crystalline lamellae formation.

AL, amorphous lamellae; CL, crystalline lamellae; SCGR, semi-crystalline growth ring; ICAGR, inter-crystalline amorphous growth ring.

Source: French (1984), reproduced with permission.


B1
A
B2
C
B3
AL
CL
AL
CL
AL
CL
SCGR
ICAGR

–2.2 on a mole basis and 0.4–1.0 on a weight basis. Manners (1989) reported A:B ratios ranging from 1.1 to 1.5, with potato amylopectin at 1.2, whereas Hizukuri (1985) reported a 0.8 A:B ratio on potato amylopectin. This discrepancy may be due to experimental errors or the different methods used. In general, amylopectins have rather more A-chains than B-chains, with ratios ranging from 1.1:1 to 1.5:1 (Manners, 1989).

2.3.2.3.2 Chain length distribution

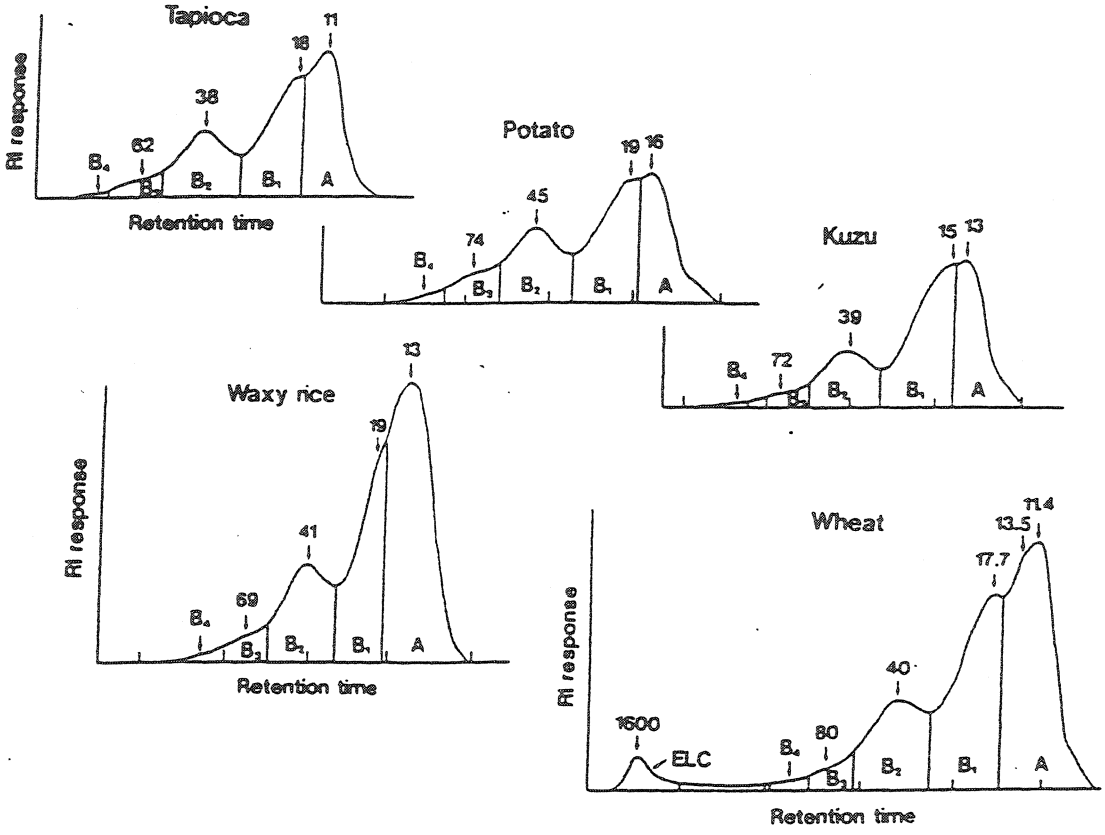
To determine its chain length distribution, amylopectin is first subjected to the action of debranching enzymes (e.g. isoamylase and pullulanase, which specifically hydrolyze the branch linkages and produce short linear chains), followed by separation techniques to determine the molecular weight distribution. (size exclusion chromatography) SEC (Hizukuri, 1985, 1986) and high-performance anion-exchange chromatography with pulsed amperometric detection (APAE-PAD) (Hizukuri, 1986; Hanashiro *et al.*, 1996; Wong and Jane, 1997) are the two basic techniques.

Extensive research has been done to study the chain length profile of amylopectin from different starch sources, such as maize (Takeda *et al.*, 1988; Jane *et al.*, 1999), barley (Tang *et al.*, 2001a, b; Song and Jane, 2000; Jane *et al.*, 1999; Yoshimoto *et al.*, 2000, 2002; MacGregor and Morgan, 1984), rice (Jane *et al.*, 1999; Hanashiro *et al.*, 1996; Takeda *et al.*, 1987b), wheat (Jane *et al.*, 1999; Shibanuma *et al.*, 1994; Franco *et al.*, 2002; Hizukuri and Maehara, 1990), tapioca (Jane *et al.*, 1999), sweet potato (Hanashito *et al.*, 1996; McPherson and Jane, 1999) and legumes (Biliaderis, 1982b; Biliaderis *et al.*, 1981; Ratnayake *et al.*, 2001; Yoshimoto *et al.*, 2001). The resulting chromatography showed that debranched amylopectin usually exhibits a trimodal or polymodal distribution pattern.

Figure 2 –8 Gel-permeation chromatograms of debranched amylopectins.

Fractions of A, B_1, B_2, B_3, B_4 and ELC (extra-long chain) are different groups of branch chains. The peak DP (degree of polymerization) is labeled on the top of each fraction.

Source: Hizukuri (1996), reproduced with permission.


Tapioca
RI response
11
18
38
62
B4
B3
B2
B1
A
Retention time
Potato
19
16
45
74
B4
B3
B2
B1
A
Kuzu
15
13
39
72
B4
B3
B2
B1
A
Waxy rice
RI response
13
19
41
69
B4
B3
B2
B1
A
Retention time
Wheat
RI response
14
13.5
17.7
40
1600
ELC
80
B4
B3
B2
B1
A
Retention time

A polymodal distribution (Fig. 2–8) shows 5 fractions, A, B_1, B_2, B_3 and B_4, representing different chain groups of the amylopectin molecule with average chain lengths ($\overline{CL}$) of 11–16, 20–24, 42–48, and 69-75, respectively. The relative lengths among B_1, B_2 and B_3 are roughly 1:2:3, implying that the A and B_1, B_2, B_3 and B_4 chains may be involved in the formation of one, two, three, and more than four clusters (Hizukuri, 1996). The A- and B_1- chains account for 80-90% (mole basis) of total chains, which represent outer short chains in a single molecule, and B_2–chains 10%, B_3–chains 1-3%, and B_4–chains 0.1-0.6%, which connect 2, 3 or more clusters (Hizukuri, 1986). Further analysis of the connection mode of branching (Hizukuri and Maehara, 1990; Hizukuri, 1996) indicated that the average span length (the number of glucose units linked through two adjacent branch points in a chain) is in the range of 3-12 and one B-chain carries 0-3 A- or other B-chains while 37% of B-chains have no A-chains and carry only B-chains. In general, the average CL for most amylopectins is in the range of 18-25, but its distribution is characteristic of starch sources. A-type starches have shorter peak DP and shorter average chain length than B- type starches. Also, A-type starches have relatively higher proportions of short chains (DP 6-12) than B-type starches and C-type starches have an intermediate amount. In addition, amylopectin from amylomaize starches have relatively longer average chain length and a higher proportion of long chains (DP≥37) compared to those from waxy and normal maize starches (Jane and Chen, 1992; Shi *et al.*, 1998; Jane *et al.*, 1999). Branch chain length distribution of amylopectin has been shown to influence starch physicochemical properties such as gelatinization temperature, pasting properties, retrogradation and acid hydrolysis (Franco *et al.*, 2002; Jane *et al.*, 1999; McPherson and Jane, 1999; Shi *et al.*, 1998; Shi and Seib, 1992, 1995; Jane and Chen, 1992). Table 2–6 summarizes the average chain length of amylopectin from some legume starches.

2.3.2.4 Double helices, crystallites and crystallinity

2.3.2.4.1 Double helices

Double helices are formed between the outer branch chains (A- and B_1- chains) of amylopectin (French, 1972). This can occur either between adjacent branch chains in the same amylopectin branch cluster or between adjacent clusters in three dimensions (Oates, 1997). Two neighboring short chains fit together compactly with the hydrophobic parts of the opposed glucose units in close contact inside the structure, and the hydroxyl groups at the outside of the double helix, for strong interchain hydrogen bonding (helical order or short range order). French and Murphy (1977) proposed the first detailed computer model (Fig. 2–9) for a starch double helix with no intra-chain hydrogen bonds. The stability of the helix is attained by interchain hydrogen bonding between hydroxyl groups at positions C2 and C6 and from van der Waals forces. The helical core is highly hydrophobic and compact so that there is no room for water or any other molecule to reside within it. Within starch granules, about 40-50% of the weight of starch chains exists in the form of double helices, with approximately half of these helical chains present in crystallites large and perfect enough to diffract X-rays (Gidley, 2001; Gidley and Bociek, 1985). The water content of starch granules has been shown to influence the content of double helices (Paris *et al.*, 1999; Bogracheva *et al.*, 2001). Bogracheva *et al.*(2001) used ^{13}C cross-polarization magnetic angle spinning NMR to study the ordered and disordered structures of selected starches from different genotypes by measuring the content of double helices at different degrees of hydration (Table 2–7). They found water contents in the 10-50% range did not influence the proportion of double helices in the starch. Decreasing the water content to 1-3% resulted in a significant decrease in the proportion of double helices, with the effect being greater in B- than in A-type starches.

Table 2 - 6
Average chain length of isolated legume amylopectins

Starch Source	Average Chain Length	Reference
Adzuki bean	21	Yoshimoto *et al.*, 2001
Adzuki bean	25 - 26	
Garbanzo bean	22	
Red kidney bean	20	
Wrinkled pea	34	Biliaderis *et al.* , 1981
Lentil	20	
Navy bean	22	
Faba bean	21	
Mung bean	23	
Smooth pea	22 - 22.4	Biliaderis *et al* ., 1981; Ratnayake *et al.* , 2002 Yoshimoto *et al.*, 2001
Pigeon pea	27	
Bonavist bean	28	Tinay *et al.* , 1983
Chick pea	29	
Chick pea (BG)	26	Madhusudhan and Tharanathan, 1996

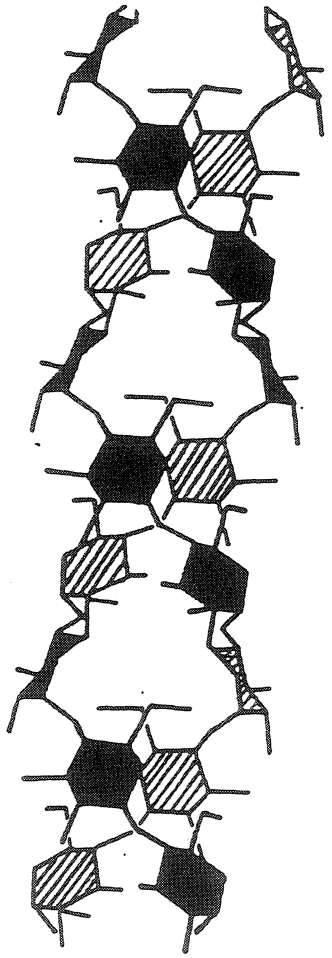
Table 2 - 7
Comparison of short-range order in starches with moderate and low water contents

Starch	Starch Type	% Proportion of Double Helices at Different Water Content(%)		Reduction in the Proportion of Double Helices after Drying, %
		Moderate	Low	
Potato	B	48.3 (15.2)[a]	23.4 (2.6)	51.6
Waxy potato	B	50.9 (13.5)	24.5 (2.0)	51.9
Pea	C	39.7 (13.3)	24.6 (1.9)	38.0
Low amylose pea	C	48.6 (12.7)	No data	No data
Maize	A	37.7 (12.3)	29.9 (0.5)	20.7
Waxy maize	A	46.1(12.2)	34.3 (0.5)	25.6
Wheat	A	31.5 (11.4)	23.7 (2.8)	24.8

a. Moisture content(%)
Adapted from Bogracheva *et al.* (2001)

Figure 2–9 Double helix model of starch chain

Source: French and Murphy (1977), reproduced with permission.



2.3.2.4.2 Crystallites and polymorphic patterns

Crystallites are formed by double helices which associate in pairs, nest together, and are stabilized by hydrogen and van der Waals forces (Oates, 1997). Three types of crystallite are known depending on the water content and the packing configuration of double helices, namely A, B, and C type. C type is a mixture of A and B type (Sarko and Wu, 1978; Blanshard, 1987; Gernat *et al.*, 1990, 1993).

The structural models of A and B type crystallites have been developed and extensively reviewed (Wu and Sarko, 1978a, b; Imberty and Pérez, 1988; Imberty *et al.*, 1988, 1991). One early model proposed by Wu and Sarko (1978a,b) is illustrated in Fig. 2–10. Both A and B type crystallite are based on parallel-stranded, right-handed double helices. The helices pack in antiparallel fashion into an orthorhombic unit cell in A type and a hexagonal unit cell in B type. Both unit cells contain twelve glucose residues. However, the B type crystallite has a more open packing structure than A type, and there are thirty six water molecules in its unit cell, whereas A type accommodates only eight water molecules. This model has been constantly reviewed and updated by other researchers. The most recent model described a face-centered monoclinic unit for the A-type crystallite instead of orthorhombic, and the helices are suggested to be left-handed instead of right-handed. Table 2–8 summarizes the structural features of A and B type crystallite unit cell.

The polymorphic patterns of starch granules can be distinguished by X-ray diffraction. Starches exhibit three types of diffraction patterns corresponding to their crystalline types (Fig. 2–11). A type appears in most cereal starches (normal maize, rice, wheat, barley, oats) and some root and tuber starches (taro, some sweet potatoes, tapioca, iris) (Zobel, 1988b; Cheetham and Tao, 1998; Hizukuri, 1996; Hoover, 2001). B type appears in tuber and root starches (potato, lily, cassava, tulip) as well as in high amylose (>40%) cereal starches (amylomaize, high-amylose barley, high-amylose rice)

(Zobel, 1988b; Hizukuri, 1996; Cheetham and Tao, 1998). The C-type, which is commonly observed in legume starches, is considered to be a mixture of A and B type in various proportions (Sarko and Wu, 1978; Blanshard, 1987; Gernat *et al.*, 1990, 1993; Hoover and Sosulski, 1991). In addition to these three types of diffraction pattern, another pattern called 'V' type was also reported, which is mainly exhibited by crystalline amylose helical inclusion compounds (Eliasson and Gudmundsson, 1996; Blanshard, 1987).

The main differences between the A and B type polymorphs are as follows: (1) packing arrangement of double helices and water content (Wu and Sarko, 1978a, b; Imberty and Pérez, 1988; Imberty *et al.*, 1988, 1991); see Fig. 2–10 and Table 2–8; (2) Chain length of amylopectin (Hizukuri, 1985, 1996; Gidley and Bulpin, 1987; Gidley and Cooke, 1991; Pfannemüeller, 1987; Hanashiro *et al.*, 1996). A type usually has a shorter average chain length, DP23 – 29; B type has a longer average chain length, DP30 – 44 (Gernat *et al.*, 1993); C type displays intermediate chain length, DP26 – 36 (Hizukuri *et al.*, 1983; Hizukuri, 1985). The difference in the average chain length between A type and B type starches can be as small as one glucose unit (Hanashiro *et al.*, 1996). (3) Branching pattern (Jane *et al.*, 1997). In A type amylopectin, the α-1, 6 branch linkages are more scattered and mainly located within the crystalline region (crystalline lamellae), whereas others are in the amorphous region (amorphous lamellae). In B type amylopectin, most of the α-1, 6 branch linkages are clustered in the amorphous region (Fig. 2-12). In A type amylopectin, due to the scattered branch points, there are likely more short A chains derived from branch linkages located inside the crystalline region, which produces an inferior crystalline structure containing α-1, 6 branch linkages and short double helices which is more susceptible to enzyme hydrolysis. Clustered branch points and relatively fewer short chains in B type amylopectin lead to the development of a superior crystalline structure, which is more resistant to enzyme attack. Additionally, other factors such as environmental temperature, the presence

of certain solutes and solvents (anions and cations), and organic molecules (alcohol, lipid) were also reported to influence starch polymorphism, but play no decisive roles (Hizukuri, 1996; Gidley, 1987).

Unlike A and B type polymorphs, which are considered to be independent from each other, C type is a mixture of A and B type in various proportions. It is still not fully understood how C type starch granules are structured. For instance, are the A and B polymorphs distributed in different granules or do they coexist within the same granule? If the two types occur within the same granule, where are they located? From the results of X-ray wide-angle scattering patterns of legume starches, Gernat *et al.* (1990) suggested the legume starches consist of starch granules of pure A type as well as of pure B type in varying proportions. However, other researchers (Bogracheva *et al.*, 1998; Buléon *et al.*, 1998b) found that all C type starch granules contain both A and B type polymorphs, instead of a mixture of pure A and B type granules. The B polymorph is present in the center of the granule and is surrounded by the A polymorph.

2.3.2.4.3 Crystallinity

Starch granules exhibit an optical birefringence pattern known as a 'Maltese cross' when viewed under polarized light, which implies that there is a high degree of molecular order within the granule (Greenwood, 1979). In fact, starch is semi-crystalline with varying polymorphic types and degree of crystallinity. The crystallinity of native starch granules usually ranges from 15 to 45% (Zobel, 1988a) (Table 2–9).

The crystallinity is exclusively associated with the amylopectin component, as crystalline lamellae are formed by tightly packed double helices of amylopectin. Cheetham and Tao (1998) have shown that the degree of starch crystallinity decreased with an increase in amylose content and

Figure 2–10 Packing arrangement of double helices of A and B type crystallite unit cell

Source: Wu and Sarko (1978a, b), reproduced with permission.

A-type

B-type

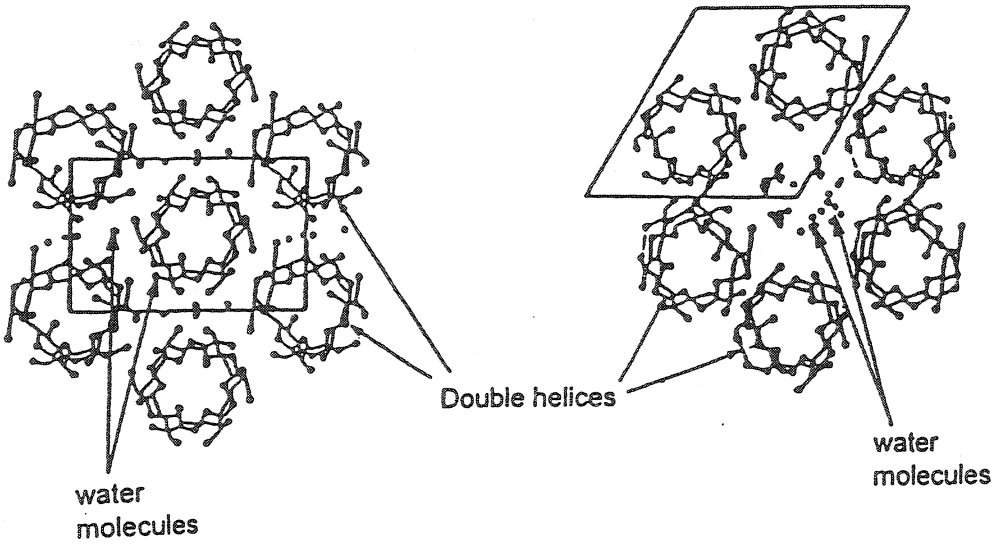


Figure 2–11 X-ray diffraction patterns of A, B, and C type starches with their characteristic d-spacing

A type: shows strong peaks at 2θ 15.27° or with a inter-crystalline spacing d=5.8 Å and 23.40° (d=3.8 Å), and an incomplete doublet at 2θ 17.05° (d=5.2 Å) and 18.1° (d=4.9 Å). The d-spacing at 4.4 Å is characteristic to amylose-lipid complex (Vasanthan and Bhatty, 1996).

B type: shows a peak at 2θ 5.52 – 5.6° (d=15.8 – 16.0 Å), a broad medium intensity peak at 2θ 15.01° (d=5.9 Å), the strongest peak at 2θ 17.05° (d=5.2 Å) and medium intensity peaks at 19.72° (d=4.5 Å), 22.22° (d=4.0 Å) and 24.04° (d-3.7 Å). There is a peak at 2θ 5° (d=17.70 Å) which is characteristic to B pattern.

C type: shows the same pattern as A type except the occurrence of the medium to strong peak at about 2θ 5.52° (d=16.0 Å)

Source: Zobel (1988b), reproduced with permission.

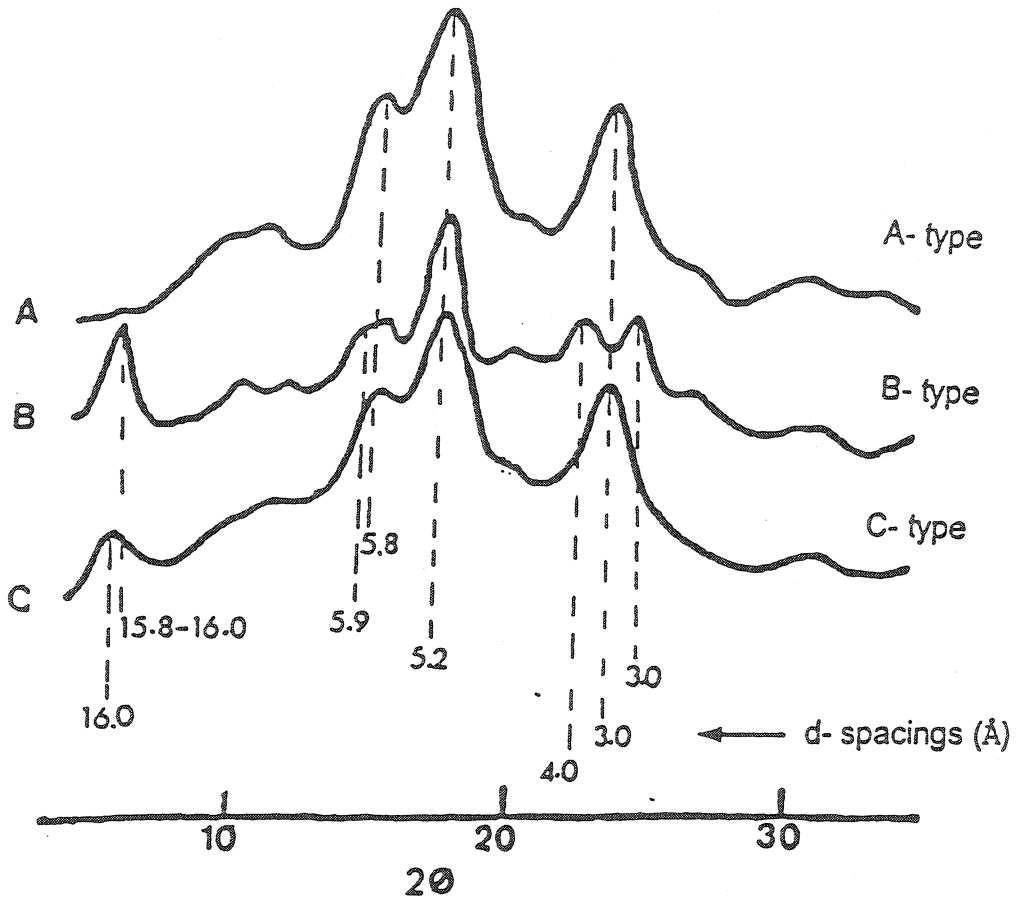
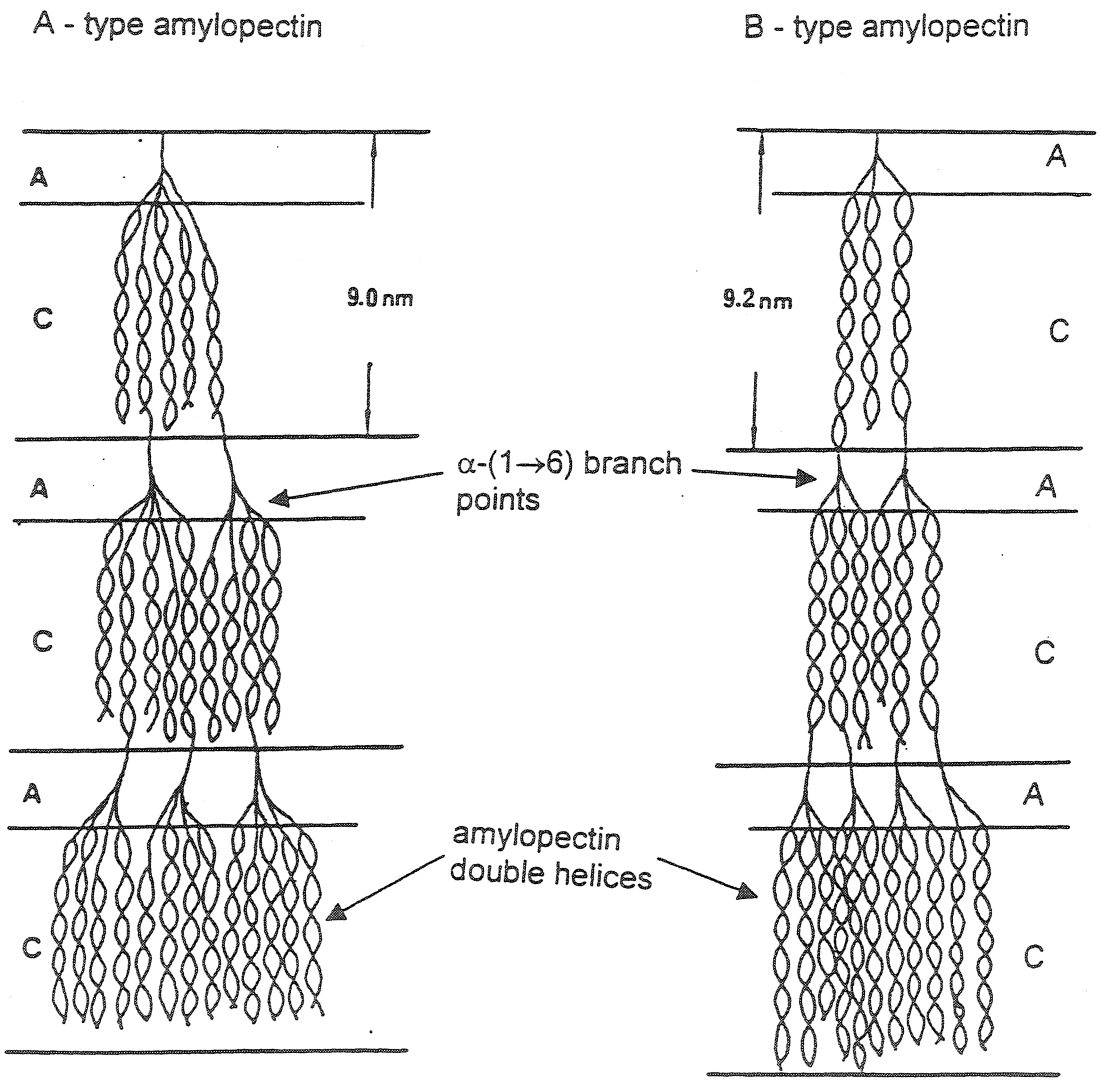

A
B
C
A- type
B- type
C- type
15.8-16.0
16.0
5.8
5.9
5.2
4.0
3.0
3.0
d- spacings (Å)
10
20
30
2Ø

Figure 2 – 12 Proposed models for branching patterns of A type (waxy maize) and B type (potato) amylopectin.

9.0nm and 9.2nm are the repeating distances of waxy maize and potato starches, respectively. The chain length between arrows stands for the length of internal long B- chains

Source: Jane *et al.* (1997), reproduced with permission.



A - amorphous C - crystalline

Table 2 - 8
Structures of 'A' and 'B' type crystalline unit cells

Parameter	A type	B type
Dimension (nm)	a = 2.124	a = 1.85
	b = 1.172	b = 1.85
	c = 1.069	c = 1.04
	γ = 123.5°	
Density	d = 1.48	
Repeat unit	Maltotriose	Maltose
Space group	B_2	$P6_1$
Geometry	Monoclinic	Hexagonal
Glucose residues	12	12
Water content	4	36
Conformation of double helices	Left-handed, double-stranded	Left-handed, double-stranded

Source: Imberty *et al.* (1991), Oates (1997)

Table 2 - 9
Crystallinity of A, B, and C starches

Starch	Crystallinity (%)	Amylose (%)	Reference
	Starches with A structure		
Oat	33	23	Zobel (1988a)
Rye	34	26	"
Wheat	36	23	"
Waxy rice	37	—	"
Sorghum	37	25	"
Rice	38	17	"
Corn	40	27	"
Waxy maize	40	0	"
Dasheen	45	16	"
Nägeli amylodextrin	48	—	Zobel (1988a)
	Starches with B structure		
Amylomaize	15 - 22	55 - 75	"
Edible canna	26	28	"
Potato	28	22	"
	Starches with C structure		
Sweet potato	38	20	"
Horse chestnut	37	25	"
Tapioca	38	18	Zobel (1988a)
Smooth pea	26 - 32	30 - 43	Davydova *et al.* (1995)
Field pea	20 - 25	42 - 44	Ratnayake *et al.* (2001)

amylopectin chain length, and it appeared to be directly proportional to the mole percent of the short chain amylopectin fraction with DP10 - 13.

Granule moisture content is another aspect related to crystallinity, but it does not affect crystal type (Cheetham and Tao, 1998). It has been reported that hydrated starches exhibited a significant increase in peak resolution and intensity of the X-ray diffractogram (Hizukuri *et al.*, 1964; Nara *et al.*, 1978; Buléon *et al.*, 1982, 1987; Veregin *et al.*, 1986; Hibi *et al.*, 1993). Peak areas at 5.5° and 25.5° in X-ray diffractograms were reported to be quite sensitive to variations in moisture content (Blanshard, 1987).

The crystallinity of starch granules can be destroyed by mechanical disruptions such as ball milling or by subjecting to high pressure at room temperature which will eventually completely destroy both the optical birefringence and the X-ray patterns (Lineback, 1984; Baldwin *et al.*, 1994).

2.4 Starch properties

2.4.1Gelatinization

Gelatinization is the collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization. The point of initial gelatinization and the range over which it occurs is governed by starch concentration, method of observation, granule type, and heterogenities within the granule population under observation (Atwell *et al.*, 1988).

Granular starch is essentially insoluble in cold water, and even when it is added to water at room temperature, little happens until heat is applied. A combination of heat and water, however, causes uncooked granules to undergo unique and irreversible changes as mentioned above, the most dramatic of which are (1) the disruption of the semi-crystalline structure, as evidenced by a loss of

birefringence; and (2) an increase in granule size, although not all granules within a given population swell at the same rate or to the same extent. As these changes are taking place, there is an attendant increase in the viscosity of the medium in which the starch is heated. When a majority of the granules have undergone this process, the starch is considered to be " pasted" or " cooked-out". In most cases, it is this pasting (i.e., viscosity-forming) ability that makes starch so functional as a food ingredient.

Various methods have been used to characterize starch gelatinization, such as polarized light microscope, Kofler hot stage microscopy, X-ray diffraction, differential scanning calorimetry (DSC), viscoamylography, NMR spectroscopy, enzymatic digestibility, and small-angle light scattering. Because not all the granules of a given starch, when subjected to water and heat, begin to gelatinize at exact by the same temperature, the gelatinization temperature is more approximately defined as a relatively narrow temperature range rather than one specific temperature. Gelatinization temperatures also vary depending on the source of the starch. In general, the gelatinization temperature of tuber and root starches such as potato and tapioca is slightly lower than that of cereal starches such as corn and wheat. The gelatinization parameters of legume starches are summarized in Table 2-10. DSC is widely used to estimate the gelatinization parameters (onset temperature T_o, peak temperature T_p, conclusion temperature T_c, gelatinization temperature range $T_c - T_o$, and gelatinization enthalpy ΔH). Noda *et al.* (1996) have postulated that DSC parameters are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (DP6 – 11), and not by the proportion of crystalline region which corresponds to the amylose to amylopectin ratio. The above authors have shown by studies on sweet potato and wheat starches, that a low T_o, T_p, T_c and ΔH reflects the presence of abundant short amylopectin chains. Cooke and Gidley (1992) have shown (^{13}C-CP-MAS-NMR and X-ray diffraction) that the enthalpy of transition is primarily due to the loss

Table 2 -10
Thermal characteristics of legume starches (DSC parameters)

Starch Source	T_o(°C)	T_p(°C)	T_c(°C)	Unspecified(°C)	ΔH(J/g)
Kidney bean[a]	62 - 67	70 - 73	76 - 79	—	15.0
Northern bean[a]	63	66	70	—	12.5
Navy bean[a]	64	68	71	—	13.4
Navy bean[c]	65.6 - 66.0	74.4 - 75.1	84.8 - 85.0	—	13.2 - 13.5
Navy bean[g]	69.8 - 71.8	76.1 - 77.5	81.7 - 85.8	—	16.3 - 19.9
Black bean[a]	62	66	70	—	12.5
Black bean[c]	62.0 - 66.9	69.9 - 76.5	82.8 - 84.2	—	12.1 - 12.9
Pinto bean[a]	72	74	79	—	16.7
Pinto bean[c]	72.0 - 72.5	75.0 - 75.5	80.5 - 81.0		15.4 - 16.2
Adzuki bean[a]	70	76	87	—	—
Smooth pea[a]	48 - 55	61 - 64	80	—	13.4
Smooth pea[c]	60.8 - 61.6	66.9 - 67.4	73.4 - 74.5	—	10.8 - 13.8
Wrinkle pea[a]	—	—	—	> 99	—
Wrinkle pea[d]	117	133	138	—	12.1
Field pea[b]	61.0 - 61.4	66.8 - 67.5	75.0 - 76.0	—	11.2 - 11.5
Chick pea[c]	59.4 - 59.7	64.7 - 67.7	71.1 - 78.2	—	9.7 - 12.4
Cowpea[e]	72.7	—	—	—	16.9
Lentil[a]	47	57	77	—	14.2
Lentil[c]	60.7 - 63.0	66.1 - 69.6	76.1 - 78.7	—	12.6 - 13.3
Lentil[f]	52.2 - 56.0	61.2 - 62.0	69.0 - 73.0	—	8.8 - 13.4

a. Hoover and Sosulski (1991), starch : water = 1 :2.
b. Ratnayake *et al.* (2001), starch : water = 1 : 3.
c. Hoover and Ratnayake (2002), starch : water = 1 : 3.
d. Colonna *et al.* (1982)
e. Chung *et al.* (1998)
f. Hoover and Manuel (1995)
g Srisuma *et al.* (1994)

of double helical order rather than the loss of crystallinity. However, Tester and Morrison (1990) have postulated that ΔH reflects the overall crystallinity (quality and amount of starch crystallites) of amylopectin. Gernat *et al.* (1993) have stated that the amount of double-helical order in native starches is strongly correlated to the amylopectin content, and that granule crystallinity increases with amylopectin content. This suggests that ΔH values should preferably be calculated on an amylopectin basis.

2.4.1.1 Mechanism of gelatinization

According to Donovan (1979) and Jenkins and Donald (1997, 1998), gelatinization in excess water is primarily a swelling driven process. In the presence of excess water, extensive hydration and swelling of the amorphous regions are considered to "strip" starch chains from the surface of crystallites, thereby disrupting crystalline order. This swelling driven process generates the DSC endotherm (referred to as G). It occurs rapidly for an individual crystallite, but over a limited temperature range for a single granule (1 - 2°C) and a wider range (10 - 15°C) for whole population of granules with endothermic enthalpy values in the range of 10 – 20J/g (French, 1984; Liu and Lelievre, 1993; Eliasson and Gudmundsson, 1996). When there is insufficient water present for all crystallites to be disrupted in this manner (usually defined as conditions in which a second endotherm is seen in DSC measurements) crystallites located in areas of locally high concentrations of water undergo "stripping", giving rise to the G endotherm; those remaining after the conclusion of this process undergo "melting" at higher temperatures, giving rise to the M1 endotherm. If the water content is reduced still further, none of the crystallites undergo the "stripping" process, and only the M1 melting endotherm is observed (Jenkins and Donald, 1998). Fig. 2–13 is an illustration of this solvation-assisted melting process.

Based on a large amount of experimental data [DSC, SAXS, SANS, dynamic mechanical analyses (DMA), optical microscopy and NMR], Waigh *et al.* (2000b) established a side-chain liquid-crystalline polymer (SCLCP) model for the structure and physical properties of starch to explain the process of gelatinization. They postulated that gelatinization is due to the inter-play between self-assembly and the breakdown of structure during heating (Waigh *et al.*, 2000a). The structural phase transitions (mesophases) can be neatly summarized by considering three order parameters: helical ordering [P_2 (cosθ)], the tendency of the helices to line up], lamellar ordering (ψ, the amplitude of a sinusoidal density modulation) and the number of helices (*h*) (Landau and Lifshitz, 1958). At low water contents (<5%, w/w) the amylopectin helices are in a glassy nematic state (Fig. 2-14a). Upon heating in a DSC a single endotherm is observed due to the helix→coil transition (Waigh *et al.*, 2000a). Intermediate water contents (>5%, <40%, w/w) have two steps in their breakdown and there are two corresponding DSC endotherms (Fig. 2-14b). The first is thought to be due to the rearrangement of dislocations between constituent amylopectin helices leading to a smectic→nematic (isotropic) transition. The second is the helix→coil transition as the amylopectin helices unwind in an irreversible manner. In excess water (40%, w/w), lamellae break up and the helix-coil transition occurs at the same point, since free unassociated helices are unstable (Fig. 2-14c), Hydrated self-assembled starches are unable to experience swelling of the crystalline growth rings before a smectic→nematic or smectic→amorphous phase transition has taken place, because unless this self assembly has occurred it implies there is insufficient water entering the granule to provoke the swelling. The self-assembly can, therefore, be viewed as a signature of solvent ingress.

Figure 2–13 Schematic representation of the possible interrelationships among the various parameters involved in phase transition phenomena of granular starch (solvation-assisted melting process).

Source: Biliaderis *et al.* (1980), reproduced with permission.

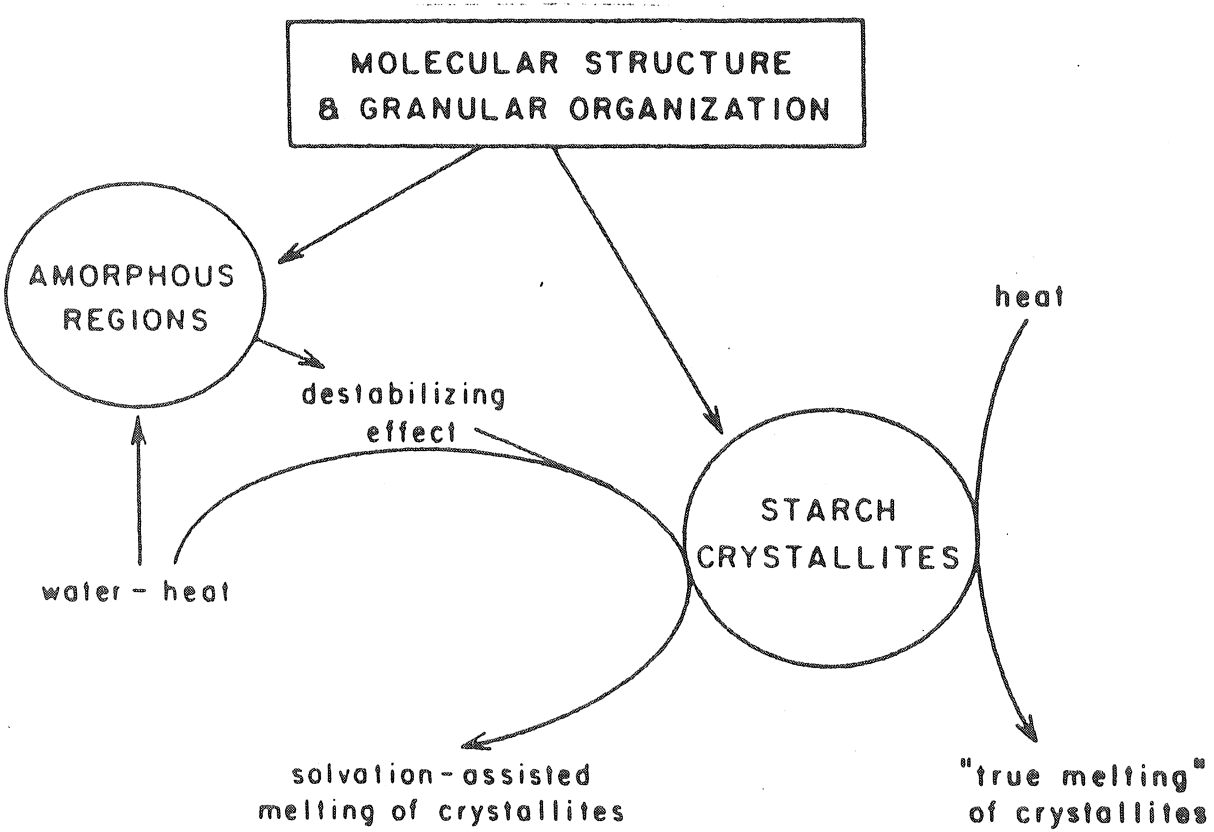

MOLECULAR STRUCTURE
& GRANULAR ORGANIZATION
AMORPHOUS
REGIONS
destabilizing
effect
heat
STARCH
CRYSTALLITES
water - heat
solvation-assisted
melting of crystallites
"true melting"
of crystallites

Figure 2-14 SCLCP model for starch gelatinization.

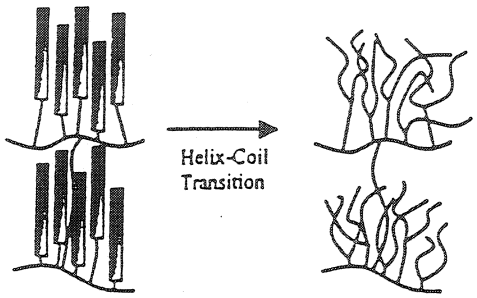
a) The single stage process in the gelatinization of starch at low water contents.

b) The two-stage process involved in the gelatinization of starch in limiting water. Two different processes are shown for A and B type starches: i) in B-type starch the intermediate phase is nematic in character; ii) and in A-type starch the intermediate phase is isotropic in character. It is proposed that the intermediate phase is determined by the length of the amylopectin helices.

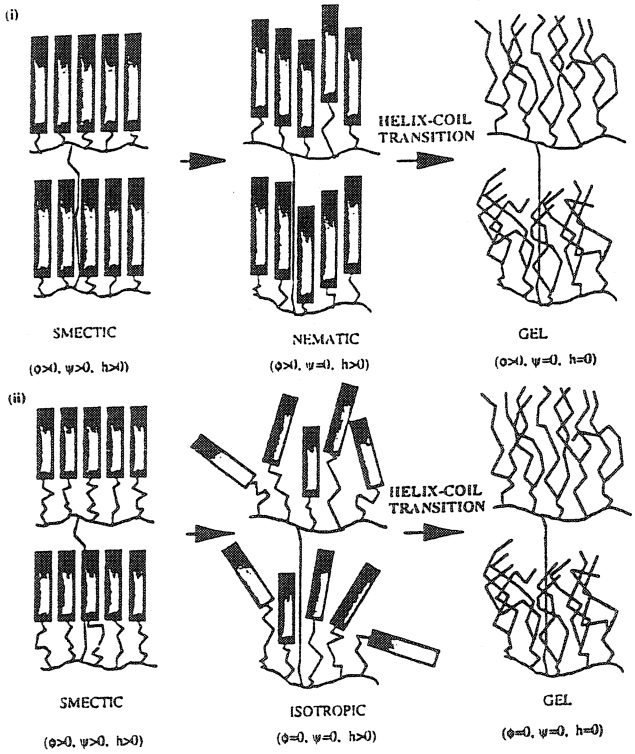
c) The two-stage process involved in the gelatinization of starch in excess water ($T_{hc}<T_{ss}$). The first stage involves a slow dissociation of the helices side-by-side. Immediately a helix-coil transition occurs as a secondary effect. Relative values of the orientational (ϕ), lamellar (ψ) and helical order parameter (h) are included. T_{hc}, temperature for the unassociated helix-coil transition of the amylopectin double helices; T_{ss}, temperature for the dissociation of helices side-by-side in their crystallites.

Source: Waigh *et al.* (2000a, b), reproduced with permission.

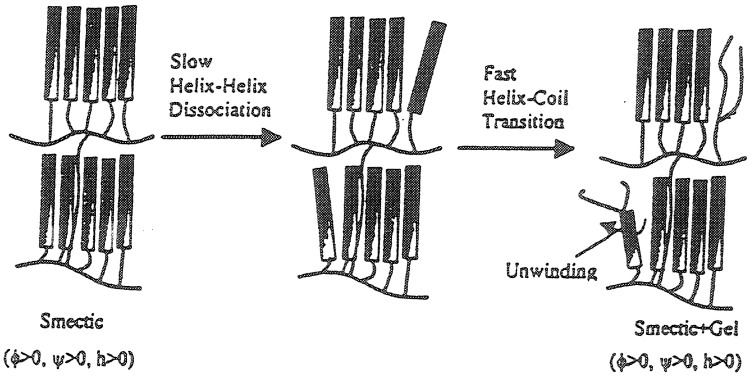
a) Low Water Content Gelatinisation



b) Intermediate Water Content Gelatinisation.



c) Excess Water Content Gelatinisation.



2.4.1.2 Factors influencing gelatinization

The gelatinization and swelling properties of starch are controlled in part by amylopectin structure (unit chain lengths, extent of branching, branching pattern, molecular weight, polydispersity and degree of phosphorylation), and starch composition (amylose/amylopectin ratio and lipid content) (Tester, 1997). However, besides these heritable traits, other factors can also influence starch gelatinization, e.g., solutes and solvents (sugars, salts, alcohols, lipids) (Evans and Haisman, 1982; Chiotelli *et al.*, 2002), physical modification (annealing and heat-moisture treatment) (Hoover *et al.*, 1993; Jacobs and Delcour, 1998; Tester and Debon, 2000), and defatting and chemical modification (acid hydrolysis, hydroxypropylation, acetylation) (Hoover *et al.*, 1988b, 1993; Hoover, 2000; Kim and Eliasson, 1993; Atichokudomchai *et al.*, 2002).

2.4.2 Retrogradation

Starch retrogradation is a process which occurs when the molecules comprising gelatinized starch begin to reassociate in an ordered structure. In its initial phase, two or more starch chains may form a simple juncture point which then may develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears (Atwell *et al.*, 1988). The molecular interactions (mainly hydrogen bonding between starch chains) develop 'B' type crystallinity on storage (Miles *et al.*, 1985a; Russell, 1987; Van Soest *et al.*, 1995) regardless of the initial crystalline pattern of the native starch. This process exerts a major and usually unacceptable influence on the texture of foods rich in starch. Starch retrogradation is the main factor in the staling of bread and other baked products (Eliasson and Gudmundsson, 1996).

Common methods used to measure retrogradation include turbidity measurement (Miles *et al.*, 1985b; Ring *et al.*, 1987; Jacobson *et al.*, 1997), DSC (Russell, 1987; Fredriksson *et al.*, 1998; Hoover

et al., 2003), rheology (I'Anson *et al.*, 1988; Mita, 1992), X-ray diffraction (I'Anson *et al.*, 1988), microscopy (Jacobson *et al.*, 1997), FT/IR (Wilson *et al.*, 1991; Van Soest *et al.*, 1995) and NMR spectroscopy (Wu and Eads, 1993). These methods provide a variety of information about the process and products of retrogradation. X-ray diffraction gives a view of the crystalline structure of the retrograded gels, DSC is well suited to follow the rate and extent of the retrogradation, while rheological methods can be used to monitor gel firmness (rigidity) on aging.

Retrogradation proceeds in two stages (Miles *et al.*, 1985a). In the first stage, the rigidity and crystallization of starch gels develop quickly as a result of amylose gelation and the increased mobility of the smaller size amylopectin fragments (Biliaderis, 1998; Zhang and Jackson, 1992). In the second stage, further crystallinity develops slowly in the amylopectin region (Biliaderis, 1998; Miles *et al.*, 1985a).

Retrogradation is influenced by starch structure (Russell, 1987; Orford *et al.*, 1987), storage temperature (Jankowski and Rha, 1986), moisture content (Longton and LeGrys, 1981; Zeleznak and Hoseney, 1986), lipids (Eliasson and Ljunger, 1988; Huang and White, 1993), sugars (Kohyama and Nishinari, 1991; Wang and Jane, 1994), salts (Ciacco and Fernandes, 1979; Bello-Perez and Paredes-Lopez, 1995), and physical and chemical modifications (Orford *et al.*, 1993; Gunaratne and Hoover, 2002; Yook *et al.*, 1993).

2.5 α-Amylolysis

2.5.1 α-Amylase

α–amylase (E.C. 3.2.1.1) is representative of a large enzyme family (glycoside hydrolase clan GH-H) known as the α–amylase family (Janeček 2000). It consists of twenty seven different enzyme specificities (Horváthová *et al.*, 2000). The α–amylase family has been defined (Takata *et al.* 1992) as

a family of enzymes that: 1) catalyze hydrolysis and/or transglycosylation at the α-1,4- and α-1,6-glucosidic linkages; 2) act with the retaining mechanism with retention of the α–anomeric configuration; 3) have four highly conserved sequence regions containing all the catalytic residues and most of the substrate binding sites; and 4) possess Asp, Glu and Asp residues as catalytic sites corresponding to Asp206, Glu230 and Asp297 of Taka-amylase A (Matsuura *et al.* 1984; Nakajima *et al.* 1986; Kuriki and Imanaka 1999).

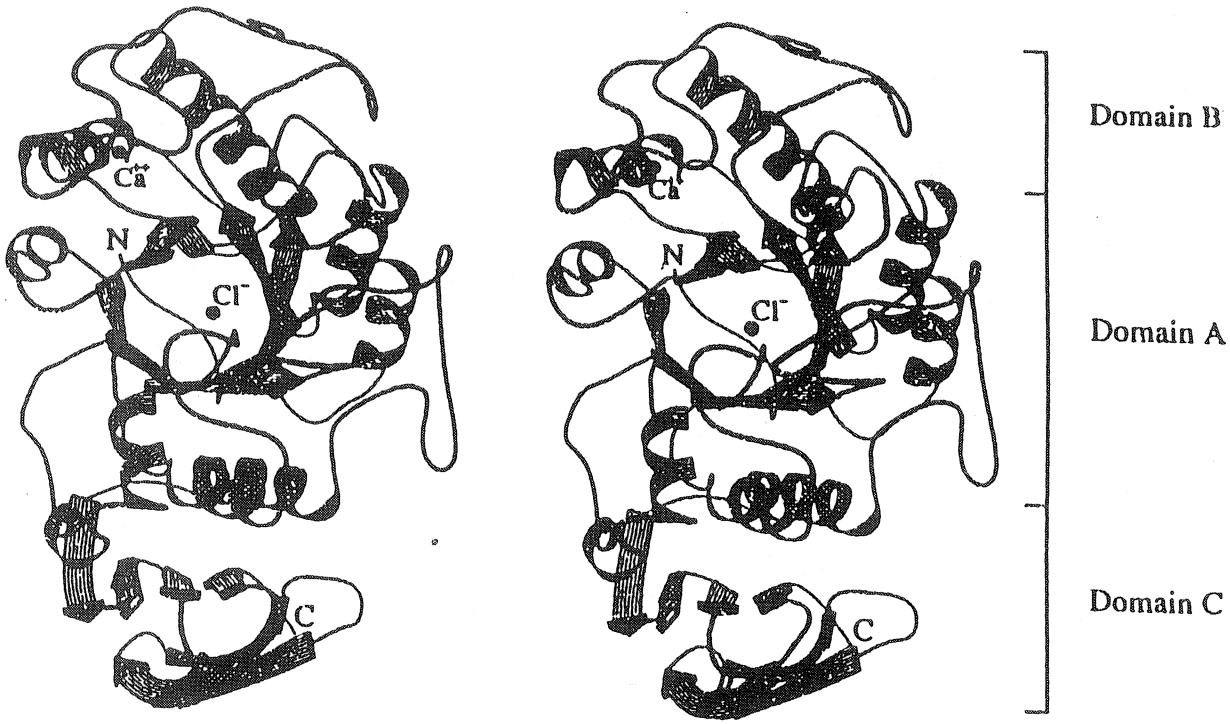
Three-dimensional X-ray structures of some α–amylases have been reported. These include: *Aspergillus oryzae* (Matsuura *et al.*, 1984; Swift *et al.*, 1991), *A. niger* (Boel *et al.*, 1990; Brady *et al.*, 1991), *Bacillus licheniformis* (Machius *et al.*, 1995), *Bacillus subtilis* (Fujimoto *et al.*, 1998), pig pancreas (Qian *et al.*, 1993; Larson *et al.*, 1994), human pancreas (Brayer *et al.*, 1995), human salivary gland (Ramasubbu *et al.*, 1996), and barley (Kadziola *et al.*, 1994). These studies have provided the overall information about α–amylase. Despite differences in their amino acid sequences, α–amylases have generally similar three-dimensional structures with three domains: domain A consisting of a central $(\beta/\alpha)_8$-barrel flanking the active site, domain B overlaying the active site from one side, and domain C consisting of a β-structure with a Greek-key motif (Fig. 2-15).

One remarkable feature of α-amylases isolated from different sources is the divergence observed in their primary sequences. For example, alignments of the sequences of animal and fungal α-amylases has found homology on the order of only ~10% between the residues present (Brayer *et al.*, 1995). Overall, studies have found only four short segments of polypeptide chain that demonstrate reasonably good homology amongst the α-amylases. The first of these segments is involved in binding a calcium ion, whereas the latter three each contain a putative active site residue (Boel *et al.*, 1990; Larson *et al.*, 1994; Qian *et al.*, 1994). In total, these represent twenty seven amino acids out of the

Figure 2–15 Schematic representation of the polypeptide chain fold of human pancreatic α-amylase.

Also indicated are the relative positions of the three structural domains featured in all α-amylases (Domain A, B, C), along with locations of the calcium and chloride binding sites. N and C indicate the terminal ends of the polypeptide chain. A central feature of this structure is the eight-stranded parallel β-barrel that forms the bulk of Domain A and is believed to contain the active site region.

Source: Brayer *et al.* (1995), reproduced with permission.


Ca++
N
Cl-
C
Ca++
N
Cl-
C
Domain B
Domain A
Domain C

~500 that make up the typical α-amylase. All other regions of the polypeptide chain sequences of animal and fungal α-amylases have essentially no homology when aligned using traditional methods based on matching schemes dependent solely on amino acid identity. Despite the results obtained from sequence alignments, recent structural studies have shown that α-amylases do have considerable similarity with regard to polypeptide chain folding, even between distantly related α-amylases. For example, Brayer *et al.*(1995) noted that overall there was ~70% topological equivalence between the animal and fungal groups of α-amylases. It seems that within the α-amylase family of enzymes, that beyond the four short segments of polypeptide chain related to active site structure, considerable flexibility is available to alter the identities of other residues to optimize enzymatic activity under the particular conditions that each α-amylase is required to function.

2.5.2 Mechanism of α-amylolysis

2.5.2.1 Cleavage of glucosidic bonds – double displacement mechanism

Throughout the α-amylase family, the enzymes are believed to have a similar mechanism of action, and so the catalytic amino residues are thought to be common to all enzymes (Svensson, 1994). Anomeric configuration is retained when the substrate is converted to product, i.e., the enzymes act on α-linkages in glucans or glucosides and yield α-linked products.

Three acidic amino acid residues (Asp, Glu, Asp) have been identified for the catalytic sites of α-amylase family enzymes based on the results obtained by X-ray crystallographic analysis (Katsuya *et al.*, 1998), chemical modification (Kuriki *et al.*, 1996), and site-directed mutagenesis (Takata *et al.*, 1994). Two distinct mechanisms have been proposed for the catalytic reaction of glycosylases: SN1 (Kaneko *et al.*, 1998) and SN2 (Tao *et al.*, 1989; Uitdehaag *et al.*, 1999). However, the SN2 mechanism, also called the double displacement mechanism, is more accepted by researchers.

The mechanism involves five steps (van der Maarel *et al.*, 2002): (1) after the substrate has bound to the active site, the glutamic acid in the acid form donates a proton to the glycosidic bond oxygen, i.e. the oxygen between two glucose molecules at subsites -1 and +1 and the nucleophilic aspartate attacks the C1 of glucose at subsite -1; (2) an oxocarbonium ion-like transition state is formed followed by the formation of a covalent intermediate; (3) the protonated glucose molecule at subsite +1 leaves the active site while a water molecule or a new glucose molecule moves into the active site and attacks the covalent bond between the glucose molecule at subsite -1 and the aspartate; (4) an oxocarbonium ion-like transition state is formed again; (5) the base catalyst glutamate accepts a hydrogen from an incoming water or the newly entered glucose molecule at subsite +1, the oxygen of the incoming water or the newly entered glucose molecule at subsite +1 replaces the oxocarbonium bond between the glucose molecule at subsite -1 and the aspartate, forming a new hydroxyl group at the C1 position of the glucose at subsite -1 (hydrolysis) or a new glycosidic bond between the glucose at subsites -1 and +1 (transglycosylation). A schematic illustration of this process is shown in Fig. 2-16. The covalently bonded intermediate has been confirmed by many researchers (Tao *et al.*, 1989; McCarter and Withers, 1996; Braun *et al.*, 1996; Mosi *et al.*, 1997; Mackenzie *et al.*, 1997a, 1997b; Uitdehaag *et al.*, 1999).

2.5.2.2 Cleavage of starch chains – multiple attack

It was proposed that α-amylases have a multiple attack mechanism when acting on starch substrates (Robyt and French, 1967) (Fig. 2–17a). In the multiple-attack mechanism, once the enzyme forms a complex with the substrate and produces the first cleavage, the enzyme remains with one of the fragments of the original substrate and catalyzes the hydrolysis of several bonds before it dissociates and forms a new active complex with another substrate molecule. It was established that the

direction of multiple attack is from the reducing end toward the nonreducing end: i.e., after the first cleavage, the fragment with the new nonreducing end dissociates from the active site, while the fragment with the newly formed hemiacetal reducing end remains associated with the active site and repositions itself to give another cleavage and the formation of maltose or maltotriose (Fig. 2–17b).

A chromatographic study of the types of low-molecular-weight products produced by porcine pancreatic and human salivary α-amylases (Robyt and French, 1967) shows that the products are maltose, maltotriose, and maltotetraose. From the investigation, it was also postulated that porcine pancreatic α-amylase has five D-glucose subsites and that the catalytic groups are located between the second and third subsites from the reducing-end subsite. The action of porcine pancreatic α-amylase on amylopectin or glycogen eventually gives a series of dextrins that contain α–D- (1 → 6) linkages. The products include tetrasaccharide, pentasaccharide, and heptasaccharide in addition to D-glucose, maltose and maltotriose (Robyt, 1984).

2.5.3 Factors influencing starch digestibility

Differences in the *in vitro* digestibility of native starches, among and within species, have been attributed to the interplay of many factors such as starch source (Ring *et al.*, 1988), granule size (Snow and O'Dea, 1981), extent of molecular association between starch components (Dreher *et al.*, 1984), amylose/amylopectin ratio (Hoover and Sosulski, 1985a), degreee of crystallinity (Hoover and Sosulski, 1985a), type of crystalline polymorphic form (A, B or C) (Jane *et al.*, 1997), distribution of B type crystallites in the granule (Gérard *et al.*, 2001), amylose-lipid complexes (Guraya *et al.*, 1997; Holm *et al.*, 1983; Nebensy *et al.*, 2002; Seneviratne and Biliaderis, 1991; Tufvesson *et al.*, 2001), physical distribution of starch in relation to dietary fiber components (Dreher *et al.*, 1984; Rao, 1969;

Figure 2 – 16 Double displacement mechanism of α-amylase

Source: Kuriki and Imanaka (1999), reproduced with permission.

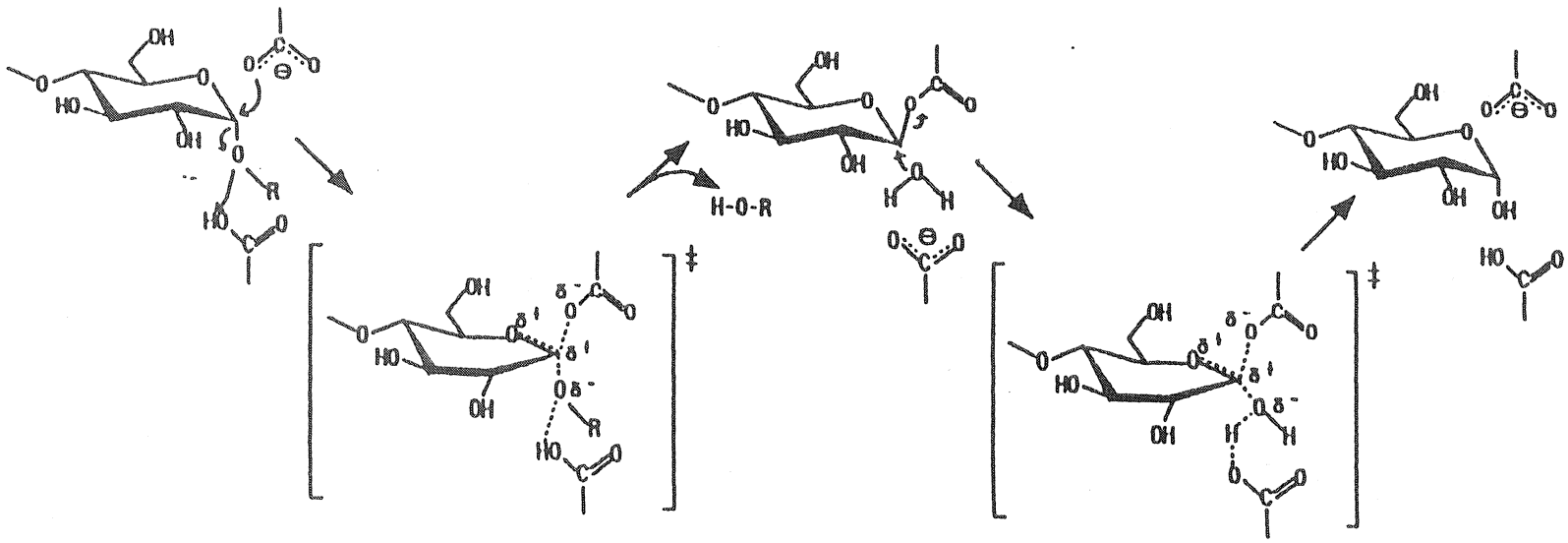

H-O-R

Figure 2–17 a) Multiple attack of α-amylase.

The arrows represent the catalytic hydrolysis of a glycosidic bond; the numbers indicate the sequence of each catalytic event. The direction of multiple attack is toward the non-reducing end.

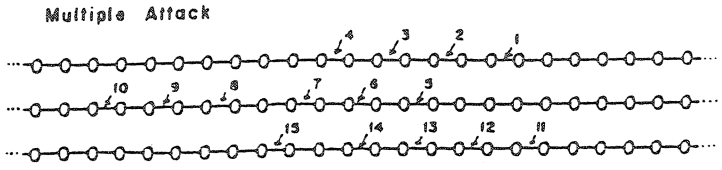
Source: Robyt and French (1970)

b) Sequence of events at the active site for multiple attack by an endo-acting enzyme.

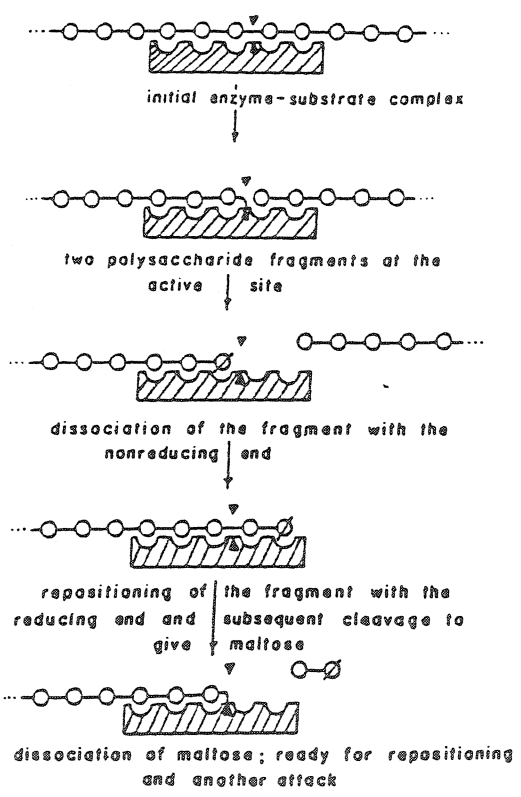
The active site is pictured here with five binding subsites and the catalytic groups located between the second and third subsites; ▲ and ▼ represent the catalytic groups; O represents a glucosyl unit; Ø, a reducing glucose unit; and —, an α–D-(1→4) glucosidic bond.

Source: Robyt (1984), reproduced with permission.

a


Multiple Attack

b


initial enzyme-substrate complex
two polysaccharide fragments at the active site
dissociation of the fragment with the nonreducing end
repositioning of the fragment with the reducing end and subsequent cleavage to give maltose
dissociation of maltose; ready for repositioning and another attack

Snow and O'Dea, 1981), antinutrients (Thompson and Gabon, 1987), α-amylase inhibitors (Lajolo *et al.*, 1991; Puls and Keup, 1973), physical insulation of starch by thick walled cells (Wursch *et al.*, 1986), porosity (Colonna *et al.*, 1988), and the influence of drying and storage conditions (Kayisu and Hood, 1979).

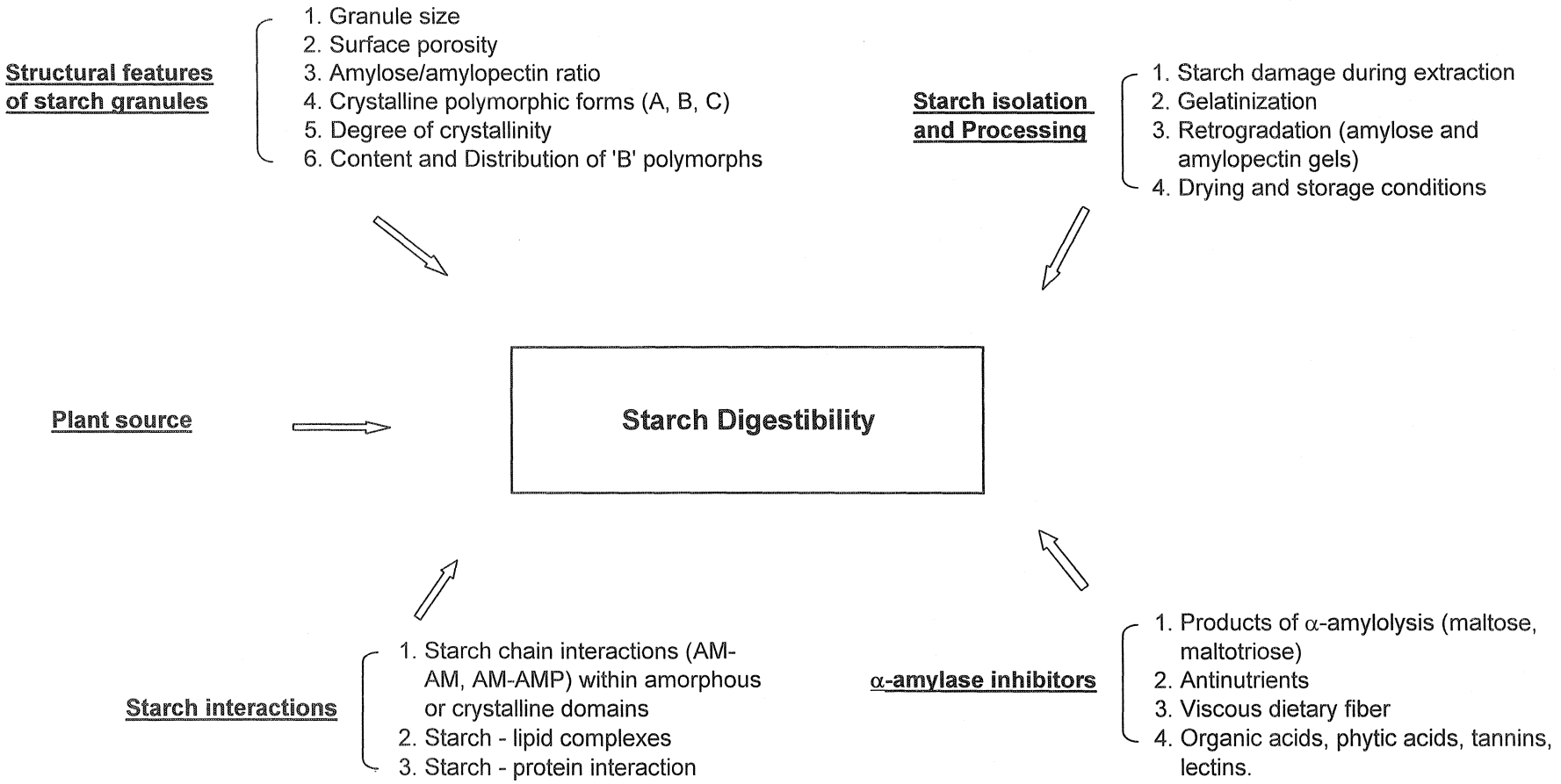
Colonna *et al.* (1992) used a mechanistic approach to analyze the steps involved in an α-amylolysis process. Four successive phases have been considered: the diffusion of the enzyme molecule towards its substrate, the porosity of starchy substrate, the adsorption of enzymes on the substrate, and finally the catalytic event. In addition, the overall hydrolysis rate was also thought to be influenced by the hydrolyzed products. Fig. 2-18 summarizes various factors involved in the kinetics of starch hydrolysis by α-amylase.

2.5.3.1 Particle size and porosity

Particle size and surface area to starch ratio play an important role in influencing the enzymatic hydrolysis rate. A larger surface area available to enzymes leads to a higher initial rate of hydrolysis. Snow and O'Dea (1981) showed that starch in cereal flours was more rapidly hydrolyzed than starch in rolled (flattened) cereals, when comparing both raw and cooked forms. Rolled cereals have a tightly packed physical form with a low surface area relative to flours. In a study on wheat (Holm *et al.*, 1985), it was shown that wet-homogenization of steam-cooked grains resulted in a higher initial hydrolysis rate with α-amylase compared with dry-milling. Microscopically, the wet-homogenized sample was more disintegrated, resulting in a greater surface area being available to the enzyme. Several researchers (Knutson *et al.*, 1982; Guraya *et al.*, 2001) have shown that the rate of α-amylase hydrolysis of starches was directly proportional to the surface area of the granules. Kong *et al.* (2003)

Figure 2-18 Factors involved in the kinetics of starch hydrolysis by α-amylase

Source: Colonna *et al.* (1992); Björck (1996); Hoover and Zhou (2003)


Structural features of starch granules
1. Granule size
2. Surface porosity
3. Amylose/amylopectin ratio
4. Crystalline polymorphic forms (A, B, C)
5. Degree of crystallinity
6. Content and Distribution of 'B' polymorphs
Starch isolation and Processing
1. Starch damage during extraction
2. Gelatinization
3. Retrogradation (amylose and amylopectin gels)
4. Drying and storage conditions
Plant source
Starch Digestibility
Starch interactions
1. Starch chain interactions (AM-AM, AM-AMP) within amorphous or crystalline domains
2. Starch - lipid complexes
3. Starch - protein interaction
α-amylase inhibitors
1. Products of α-amylolysis (maltose, maltotriose)
2. Antinutrients
3. Viscous dietary fiber
4. Organic acids, phytic acids, tannins, lectins.

studied porcine pancreatic α-amylase activity on native starch granules from potato, maize and rice, and they found that the reciprocal of initial velocity was a linear function of the reciprocal of surface area.

Specific area and porosity are considered as physicochemical factors responsible for differences in susceptibility of native starch granules (McGregor and McGregor, 1985; Colonna *et al.*, 1988). It has been speculated (Leach and Schoch, 1961; Gallant *et al.*, 1972) that amylases hydrolyze native starch granules by entering the granule through pores or a loose sponge-like structure that permits the enzyme molecules to penetrate into the granule and hydrolyze the starch chains. These pores might be inherent properties of the various starches and the number may vary according to the type of starch. The presence of pores has been observed by Fannon *et al.* (1992) for maize, sorghum and millet starches and by Baldwin *et al.* (1998) for potato, rice and wheat starches. Fannon *et al.* (1993) further pointed out that the pores present on the external surface of sorghum starch granules are openings to serpentine channels that penetrate into the granule interior.

2.5.3.2 Amylose/amylopectin ratio

The amylose/amylopectin ratio is higher in legume starches than in "common" varieties of cereal or tuber starches (Eliasson, 1988). Due to the low glycaemic index (GI) reported for most legume products, this has focused interest on potential varietal differences in amylose content.

Among cereals (e.g., rice, corn, and barley) the amylose/amylopectin ratio may differ considerably among genotypes. In some studies on rice (Goddard *et al.*, 1984; Juliano and Goddard, 1986; Miller *et al.*, 1992), a higher amylose content was shown to lower metabolic response. In fact, according to Miller *et al.* (1992), only high-amylose varieties of rice are potentially useful in low-GI diets. By exchanging high-amylose corn flour for ordinary corn flour (70%) in glucose and insulin

responses were significantly reduced in healthy subjects (Granfeldt *et al.*, 1995). Sticky rice, with a lower amylose content, was hydrolyzed very rapidly *in vitro*, producing a hydrolysis graph similar to that with white bread (Granfeldt *et al.*, 1992). Also, the metabolic responses and *in vitro* rate of starch hydrolysis of gels made from wheat, manihot, and smooth pea starch were inversely related to the amylose content (17–35%) (Bornet *et al.*, 1989).

Inclusion of high-amylose corn starch (70% amylose) into products has been shown to lower metabolic responses compared with products based on low-amylose starch. As a consequence, Behall *et al.* (1988) reported reduced postprandial responses of glucose and insulin in healthy subjects following ingestion of crackers made from high-amylose corn starch compared with a corresponding product made from low-amylose starch. A beneficial effect of incorporating autoclaved, high-amylose corn starch into products was further reported by van Amelsvoort and Weststrate (1992) in healthy subjects. In both investigations, the effect on insulin was most pronounced, which is in accordance with data on high-amylose rice starch (Goddard *et al.*, 1984).

2.5.3.3 Starch interactions/physical structure

Interactions between starch and protein can greatly influence the α-amylolysis rate. It has been demonstrated that the protein matrix, in cereal (Holm and Björck, 1988; Holm *et al.*, 1989; Jenkins *et al.*, 1987a) as well as in legume products (Tovar *et al.*, 1990), limits the accessibility of starch to amylase. By deproteinizing pasta, the rate of *in vitro* amylolysis increased (Colonna *et al.*, 1990). Differences in the *in vitro* rate of amylolysis between processed wheat samples were evened out following preincubation with pepsin (Holm and Björck, 1988; Holm *et al.*, 1989). The *in vitro* procedure, which included pepsin, allowed closer prediction of glycemic response in rats, suggesting

that at least some protein-starch interactions are broken under physiological conditions (Holm *et al.*, 1989).

In pasta products, gluten forms a viscoelastic network that surrounds the starch granules, thus restricting starch swelling and leaching of starch during boiling (Colonna *et al.*, 1990; Pagani *et al.*, 1986). As to the cause of the slow-release starch features of pasta, the limited swelling of starch granules may reduce the availability to amylases. However, as judged from the prominent increase in the rate of amylolysis even at pregelatinized stages of granule crystallinity (Holm *et al.*, 1988), differences in the extent of swelling of gelatinized granules might not account for the lowered availability observed. The presence of a glutinous phase, although available to preteolytic enzymes, will possibly release the starch substrate more gradually to amylolytic attack. According to Jenkins *et al.* (1987a), protein-starch interaction also reduces the availability of the starch in bread products, and a bread made from gluten-free flour elicited a higher glucose response than an ordinary wheat bread.

Other forms of starch interactions involve formation of amylose-lipid complexes and interactions between starch molecules. Amylose-lipid complexation affected the enzyme susceptibility of sago starch by reducing starch granule swelling (thus providing less opportunity for enzyme access to the granule interior and less leaching of amylose from the granules) and by increasing resistance to digestive enzymes (Cui and Oates, 1999). Amylose complexed with lysolecithin was more slowly digested and absorbed from the rat small intestine, and produced less pronounced postprandial glycemia than "soluble" amylose (Holm *et al.*, 1983). This reduction is noteworthy, since the soluble amylose reference also can be expected to be less readily available to amylases due to its disposition to retrograde. In products based on starches in which amylose is the minor starch component, amylose retrogradation and/or formation of amylose-lipid complexes is probably most efficient in reducing the enzymic availability if the amylose involved is enriched on surfaces, thus encapsulating the bulk of

starch, rather than being evenly distributed. Such interactions may explain the improvement in product characteristics of rice seen in some studies as a result of parboiling. The low glycemic responses reported for products based on high-amylose starches might, however, be related to retrogradation of amylose. Although high-amylose starch granules do not swell under "ordinary conditions for food preparation", following the disappearance of the crystalline granule structure, amylose molecules could still interact, leading to a reduction in the overall enzymic availability of starch. As to the effect of retrogradation of amylopectin, even significant staling of starch in bread did not reduce the rate of *in vitro* amylolysis (Siljeström *et al.*, 1988).

2.5.4 Hydrolysis of legume starches

Native legume starches have been found to be more digestible than native potato or high amylose maize starch, but less digestible than native cereal or cassava starch (Dreher *et al.*, 1984; Frins *et al.*, 1998; Hoover and Sosulski, 1985a; Ring *et al.*, 1988; Socorro *et al.*, 1989; Tovar *et al.*, 1991). Hoover and Sosulski (1985a) have shown that during a 6h digestion with porcine pancreatic α-amylase, maize starch was hydrolyzed to the extent of 75%, whereas, at the same enzyme concentration, the corresponding value for legume starches belonging to the biotype *Phaseolus vulgaris* ranged from 25 to 35%.

The reduced bioavailability of legume starches has been attributed to the presence of intact tissue/cell structures enclosing starch granules, higher levels of amylose (30 – 65%), high content of viscous soluble dietary fiber components, the presence of a large number of antinutrients, 'B' type crystallites and strong interactions between amylose chains (Deshpande and Cheryan, 1984; Hoover and Sosulski, 1985a; Siddhuraju and Becker, 2001; Tovar *et al.*, 1991; Wursch *et al.*, 1986). Table 2–11 presents the *in vitro* amylolysis of legume starches by α-amylase from different origins. It is

difficult to rank the legume starches with regard to their susceptibility towards α-amylase, due to differences in enzyme concentration, time of hydrolysis and source of α-amylase. Furthermore, the data presented in Table 2–11 have been on a single cultivar. Therefore, it is difficult to ascertain whether the reported extent of starch hydrolysis is truly representative of the particular species.

There is growing interest in the application of legume starches as resistant starch. Resistant starch is defined as the sum of starch and starch degradation products not digested in the small intestine of healthy individuals. It is subdivided into four categories depending on the cause of resistance (Englyst *et al.*, 1992; Eerlingen *et al.*, 1993): RS_1, physically inaccessible starch due to entrapment in a nondigestible matrix; RS_2, raw starch granules with crystallinity; RS_3, retrograded amylose; and RS_4, chemically modified starch slowly digestible starch (SDS) and RS are of particular interest because of their potential health benefits to humans. A high proportion of SDS relative to rapidly digestible starch (RDS) in a starchy food indicates a food with a low glycemic index. Foods with a low glycemic load are thought to be beneficial for all individuals, especially for type II diabetics (Englyst *et al.*, 1999; Björck *et al.*, 2000; Roberts, 2000; Roberts *et al.*, 2000; Jenkins *et al.*, 2001).

Raw and processed legumes have been shown to contain significant amounts of RS in comparison to cereal and potatoes (Bednar *et al.*, 2001; Björck *et al.*, 1994; Bravo *et al.*, 1998; Elmstahl, 2002; Garcia-Alonso *et al.*, 1998; Lehmann *et al.*, 2003; Lintas and Cappelloni, 1992; Marlett and Longacre, 1996; Osorio-Diaz *et al.*, 2002; Periago *et al.*, 1997; Rosin *et al.*, 2002; Tovar and Melito, 1996; Tovar *et al.*, 1992a, b; Truswell, 1992; Velasco *et al.*, 1997). For this reason the ingestion of legumes results in reduced glycemic and insulinemic postprandial responses in comparison to cereals or potatoes (Jenkins *et al.*, 1982, 1987b; Tovar *et al.*, 1992b).

Table 2 - 11
In vitro digestibility of native legume starches

Starch Source	Source of a-amylase	Reaction Time(h)	Degree of hydrolysis(%)
Smooth pea (*Pisum Sativum* L.)	Porcine Pancreatic	24	18.2 - 22.2
	Porcine Pancreatic	29	91
	Bacillus Sp.	29	78
	Aspergillus Fumigatus	29	100
Wrinkled pea (*Pisum Sativum* L.)	Porcine Pancreatic	29	72
	Bacillus Species	29	66
	Aspergillus Fumigatus	29	77
Grass pea (*Pisum sativum* L.)	Porcine Pancreatic	24	22
Green pea (*Pisum sativum* L.)	Porcine Pancreatic	24	16
Beach pea (*lathyrus maritimus* L..)	Porcine Pancreatic	24	35
Lentil (*lens culinaus* L.)	Porcine Pancreatic	24	14.5 - 35.5
Mung bean (*Phaseolus aureus*)	Porcine Pancreatic	24	71.1
Lima bean (*Phaseolus lunatus*)	Porcine Pancreatic	6	25
Lablab bean (*Lablab purpureus*)	*Bacillus* Subtilis	2	30
Pinto bean (*Phaseolus vulgaris*)	Porcine Pancreatic	1	62
	Porcine Pancreatic	6	25.2
Navy bean (*Phaseolus vulgaris*)	Porcine Pancreatic	6	32
Northern bean (*Phaseolus vulgaris*)	Porcine Pancreatic	6	29
Black bean (*Phaseolus vulgaris*)	Porcine Pancreatic	6	34.8
	Porcine Pancreatic	3	49.5
Kidney bean (*Phaseolus vulgaris*)	Porcine Pancreatic	6	31.4
Moth bean (*Phaseolus acontifolius*)	Porcine Pancreatic	NA	25.4 - 28.2
	Human Salivary	1.6	30.2
Tepary bean (*Phaseolus acutifolius*)	Porcine Pancreatic	2	8
Yam bean (*Sphenostylis stenocarpa*)	Saliva	1	18
Chick pea (*Cicer arientum*)	Porcine Pancreatic	24	60
	Human Salivary	2	15
Horse gram (*Dolichos biflorus*)	Saliva	2	10.2
Cow pea (*Vigna Sinensis*)	Saliva	2	10.8

Source: Hoover and Zhou (2003)

Chapter 3 Materials and Methods

3.1 Materials

Black bean (*Phaseolus vulgaris* L.) cultivars (*CDC Nighthawk, Black Jack*); pinto bean (*Phaseolus vulgaris* L.) cultivars (*Othello, Sierra*); lentil (*Lens culinaris* Mekik) cultivars (*CDC Rrobin, CDC Redwing*); smooth pea (*Pisum sativum* L.) cultivars (*CDC Mozart, CDC Sonata*) and wrinkled pea (*Pisum sativum* L.) were obtained from the Crop Development Center, University of Saskatchewan, Saskatoon, Canada. Crystalline porcine pancreatic α-amylase (E.C. 3.2.2.1, type 1A) was purchased from Sigma Chemical Co., (St. Louis, MO, USA). Potato starch and waxy corn starch were gifts from National Starch and Chemical Co., Bridgewater, NJ, USA. All other chemicals and solvents were of ACS-certified grade.

3.2 Methods

3.2.1 Starch isolation

Starch was isolated from legume seeds by the procedure of Hoover and Sosulski (1985a). Seeds (200 g) were steeped in 300 mL of 0.01% (w/v) sodium metabisulfite for 24 h at ambient temperature (20-25°C). The swollen seeds were thoroughly washed with water, peeled and homogenized in a Commercial Waring Blender (Dynamics Corporation of America, New Hartford, CT, USA) for 90 sec. The homogenate was then filtered (under vacuum) through a double-layer of cheesecloth. The filtrate was collected and left to settle for 2 h, then the supernatant was removed by a siphoning tube. The sediment was suspended in excess 0.2% (w/v) NaOH and after standing for 12 h the supernatant was removed. The sedimentation procedure was repeated thrice. The final sediment was suspended in distilled water and passed through a 70 μm polypropylene filter cloth under vacuum. The filtrate was

allowed to settle for 2h and the supernatant was removed, again this procedure was repeated three times. Finally, the slurry was neutralized to pH 7.0 by HCl and passed through a double-layer of Whatman No.4 filter paper, and the filter cake oven dried at 30°C for 24h in a Fisher Isotemp 615G forced air oven (Fisher Scientific, Nepean, ON, Canada). The dried starch cake was carefully crushed and sieved (250 μm test sieve, Fisher Scientific Company, USA) to obtain a free flowing powder, which was weighed and the yield was calculated as the percentage of the initial seeds weight.

3.2.2 Granule morphology

The granule surface was studied by scanning electron microscopy. Starch samples were mounted on circular aluminum stubs with double sticky tape and then coated with 20 nm of gold and examined and photographed in a Hitachi scanning electron microscope (S570, Nissei Sangyo, Inc., Rexdale, ON, Canada) at an accelerating potential of 5 kV. The size and shape of native starches were examined by a Leica Gallen III microscope. The range of granule size was determined by measuring the length and width of 40 granules from a 1.0% starch suspension at 10 × 100 magnification with an eye-piece micrometer.

3.2.3 Compositional analyses

3.2.3.1 Moisture content

Quantitative estimation of moisture was performed according to standard AACC (American Association of Cereal Chemists, 1984) procedures. Pre-weighed (4-5 g) starch samples were dried in a forced air oven (Fisher Isotemp 615G, Fisher Scientific, Nepean, ON, Canada) at 130°C for 1 h. The sample was then removed and cooled in a desiccator. The moisture content was calculated as the percentage weight loss of the sample.

3.2.3.2 Ash content

Pre-weighed (5±0.01 g) samples were transferred into a clean, dry porcelain crucible, and ignited over a flame until thoroughly carbonized. Then the sample was placed in a pre-heated (525°C) muffle furnace (Lab Heat – Blue M model M30A-1C, Blue M Electric Co., Blue Island, IL, USA) and allowed to stand until it became a cotton-like substance and free of carbonaceous matter (~12 h). The sample was cooled to room temperature in a desiccator and weighed. The ash content was calculated as the percentage weight loss of the sample (AACC, 1984).

3.2.3.3 Nitrogen content

Nitrogen content was determined by the micro-Kjeldahl method. Samples (0.3 g, db) were weighed on nitrogen free paper and placed in digestion tubes on a Buchi 430 digester (Buchi Laboratorimus-Technik AG, Flawill/Schweiz, Switzerland). The catalyst (two Kjeltab M pellets) and 20 mL of concentrated sulfuric acid were added to each tube and the sample was digested until a clear yellow solution was obtained. The digested samples were then cooled, diluted with 50 mL of distilled water, 100 mL of 40% (w/v) NaOH was then added, and the released ammonia was steam distilled into 50 mL of 4% (w/v) boric acid (H_3BO_3) containing 12 drops of end-point indicator (N-point indicator, EM Science, NJ, USA) using a Buchi 321 distillation unit until 150mL of distillate was collected. The amount of ammonia in the distillate was determined by titrating against 0.05N sulfuric acid (AACC, 1984). Percentage nitrogen was calculated as:

$$\text{Nitrogen (\%)} = \frac{(\text{Volume of acid} - \text{Blank}) \times \text{Normality of acid} \times 14.0067 \times 100}{\text{Sample weight (mg)}}$$

3.2.3.4 Lipid content

Different categories of lipid were extracted from starch by the following procedures and the amounts of extracted lipid were expressed as percentages of the initial starch sample weight.

3.2.3.4.1 Surface lipids

Surface lipids were extracted at room temperature (25 - 27°C) by mixing starch (5 g, db) with 100mL of 2:1 (v/v) chloroform-methanol under vigorous agitation in a wrist action shaker for 1h. The solution was then filtered (Whatman No.4 filter paper) into a round bottom flask and the residue was washed thoroughly with a small amount of the above chloroform-methanol solution. The solution was then evaporated to dryness using a rotary evaporator (Rotavapor -R110, Buchi Laboratorimus – Technik AG, Flawill/Schweiz, Switzerland). The crude lipid extracts were purified by the method of Bligh and Dyer (1959) before quantification. The starch residue was saved for bound lipid extraction.

3.2.3.4.1.1 Bligh and Dyer (1959) method of lipid purification

The crude lipid from the above extract was purified by extraction with chloroform-methanol-water (1:2:0.8, v/v/v) and by forming a biphasic system (chloroform-methanol-water, 1:1:0.9, v/v/v) by the addition of chloroform and water at room temperature (25-27^{o}C)in a separation funnel. The chloroform layer was then diluted with benzene and brought to dryness using a rotary evaporator followed by drying at 60°C for 1h in a forced-air oven. The dried lipid was cooled to room temperature in a desiccator.

3.2.3.4.2 Bound lipids

Bound lipid was extracted using the residue left after surface lipid extraction. The residue was refluxed with 3:1 (v/v) n-propanol-water in a soxhlet apparatus at 85°C for 7 h (Vasanthan and Hoover, 1992). The extracted solution was evaporated using a rotary evaporator. The crude lipid extract was purified by the method of Bligh and Dyer (1959) before quantification.

3.2.3.4.3 Total lipids

Total starch lipid was determined by hydrolyzing starch (2 g, db) with 25 mL of 24% (v/v) HCl at 70-80°C for 30 min. The hydrolyzate was extracted three times with n-hexane. The extract was evaporated to dryness in a rotary evaporator. The crude lipid extract was purified by the method of Bligh and Dyer (1959) before quantification.

3.2.3.5 Amylose content

Apparent and total amylose content were determined as described by Hoover and Ratnayake (2001).

3.2.3.5.1 Apparent amylose

Starch (20 mg, db) was accurately weighed into a round bottom screw cap tube, then 8 mL of 90% dimethylsulfoxide (DMSO) was added to the tube. The contents were mixed vigorously for 2min using a vortex mixer followed by heating in a water bath (PolyScience, Model 2L-M, PolyScience, Niles, IL, USA) at 85°C for 15 min with intermittent shaking. The tube was then allowed to cool to room temperature (~45 min) and then diluted to 25 mL in a volumetric flask. 1 mL of the diluted solution was mixed with water (40 mL), 5 mL of I_2/KI solution (0.0025M I_2 and 0.0065M KI mixture)

was added, and the final volume was adjusted to 50 mL in a volumetric flask. After 15 min (for color development), the absorbance was read at 600 nm using a UV-visible spectrophotometer (LKB Novaspec-4049 spectrophotometer, LKB Biochrom Ltd., Cambridge, England). In order to avoid over-estimation of amylose content (due to complex formation between I_2 and the long outer branch chains of amylopectin), amylose content was calculated from a standard curve prepared using mixtures of pure potato amylose and amylopectin (over the range 0-100% amylose and 100-0% amylopectin) (Fig. A-1 in Appendix).

3.2.3.5.2 Total amylose

Starch samples were defatted by extracting in a Soxhlet extractor (85°C) with 3:1 (v/v) n-propanol-water for 7h prior to the determination of total amylose content by the above procedure.

3.2.4 Starch damage

Starch damage was estimated following the AACC (1984) standard procedures. Starch samples (1g, db) were digested with fungal α-amylase from *Aspergillus oryzae* (0.05 g) having a specific activity of 50-200 units/mg in a water bath (PolyScience waterbath, PolyScience, Niles, IL, USA) at 30°C for 15 min. At the end of the incubation, the enzyme action was terminated by adding 3.68N sulfuric acid (3 mL) and 12% (w/v) sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) (2 mL), respectively. The mixture was allowed to stand for 2min and then filtered through a Whatman No. 4 filter paper. Aliquots (2 mL) of the filtrate were mixed with 2 mL of 3,5 – dinitrosalicylic (DNS) acid and then heated in a boiling water bath for 5 min. The reaction mixture was chilled using an ice bath and diluted with 8 mL of distilled water. The absorbance was measured at 540 nm against a blank (Bruner, 1964) (the details of the procedure are outlined in 3.2.4.1). A calibration curve (Fig. A-2 in Appendix) was

established with maltose (to calculate the maltose equivalents in the digest) and the percentage starch damage was calculated using the following equation:

$$\text{Starch damage (\%)} = (M \times 1.64) / (W \times 1.05) \times 100$$

M: mg maltose equivalents in the digest.

W: mg (db) of starch

1.64: the reciprocal of the mean percentage maltose yield from starch (an empirical factor which assumes that under the conditions of the experiment, the maximum degree of hydrolysis is 61%).

1.05: molecular weight conversion of starch to maltose

3.2.4.1 Determination of reducing sugar content (Bruner, 1964)

Sample solution (2 mL) was pipetted into a screw cap tube, followed by 2 mL of 3,5-dinitrosalicylic acid (DNS) solution (20 g of DNS dissolved in 700 mL of 1N NaOH). The mixture was stirred well to dissolve the DNS and then diluted to 1 L with distilled water and filtered through a double-layer of Whatman No. 1 filter paper. The mixture was heated in a boiling water bath for 5 min for color development. The tube was then cooled in an ice bath for 10 min, and then 8 mL of distilled water was added to make the total volume to 12 mL. The absorbance was read at 540 nm using a UV-visible spectrophotometer (LKB Novaspec-4049 spectrophotometer, LKB Biochrom Ltd., Cambridge, England) against a reagent blank (25°C). Standard curves were established by preparing a series of mixtures with known amounts of maltose (Fisher Scientific, Fair Lawn, NJ, USA) (Fig. A-2 in Appendix).

3.2.5 Swelling factor (SF)

The SF of the starches at 80°C in excess water was measured according to the method of Tester and Morrison (1990). Starch samples (50 mg, db) were weighed into a screw cap tube, 5 mL of water was added, and the tube was heated in a shaking water bath at 80°C for 30 min. The tube was then cooled to 20°C immediately on ice, 0.5 mL of blue dextran (MW 2,000,000) was added and mixed well. The tube was then centrifuged at 2000 r.p.m for 5 min and the absorbance of the supernatant was measured at 620nm using a UV-visible spectrophotometer against a reference without starch. This method measures only intragranular water and hence is regarded as the true SF at a given temperature.

Calculation of SF was based on starch weight corrected to 10% moisture, assuming a density of 1.4 mg/mL.

Free or interstitial plus supernatant water (FW) is given by:

$$FW = 5.5\ (A_r/A_s) - 0.5$$

Where A_r and A_s are the absorbances of the reference and sample, respectively.

The initial volume of starch (V_o) of weight W (in mg) is

$$V_o = W/1{,}400$$

And the volume of absorbed intragranular water (V_1) is thus:

$$V_1 = 5.0 - FW$$

Hence the volume of the swollen starch granule (V_2) is:

$$V_2 = V_o + V_1$$

And $SF = V_2 / V_o$

This can also be expressed by the single equation:

$$SF = 1 + \{(7700 / W) \times [(A_s - A_r) / A_s]\}$$

The coefficient of variation of the method was generally less than 1%.

3.2.6 Extent of amylose leaching (AML)

Native starches (20 mg, db) in water were heated at 80°C in volume-calibrated sealed tubes for 30min. The tubes were then cooled to ambient temperature (25-27°C) and centrifuged at 2000 r.p.m for 10 min. The supernatant liquid (1 mL) was withdrawn and its amylose content determined as described by Hoover and Ratnayake (2001). Amylose leaching was expressed as percentage of amylose leached per 100 g of starch.

3.2.7 Differential scanning calorimetry (DSC)

Gelatinization parameters of native and enzyme treated residues were measured and recorded on a Seiko DSC 210 (Seiko Instruments Inc., Chiba, Japan) differential scanning calorimeter equipped with a thermal analysis data station and data recording software. Water (11μL) was added with a microsyringe to starch (3.0 mg) in the DSC pans, which were then sealed, weighed, and allowed to stand for 24 h before DSC analysis. The scanning temperature range and the heating rate were 20-120°C and 10°C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as the reference. The transition temperatures reported are the onset (T_o), peak (T_p), and conclusion (T_c) of the gelatinization endotherm. The enthalpy of the gelatinization (ΔH) was estimated by integrating the area between the thermogram and a base line connecting the points of onset and conclusion temperature, and was expressed in terms of mJ/mg starch (Fig. A-4 in Appendix). All DSC experiments were performed in triplicate.

3.2.8 X-ray diffraction

X-ray diffractograms of native and enzyme hydrolyzed starches were obtained with a Rigaku RU 200R X-ray diffractometer (Rigaku-Denki Co., Tokyo, Japan) under the following operating

conditions as: target voltage – 40 KV, current – 100 mA, aging time – 5 min, scanning range – 3-35°, scan speed – 2.000°/min, step time – 4.5 s, divergence slit width – 1.00, scatter slit width – 1.00 and receiving slit width – 0.60.

The moisture contents of all starch samples were adjusted to ~19% by being kept in a desiccator over saturated $BaCl_2$ solution (25°C, a_w=0.9) for about 1 week (Barron *et al.*, 2000).

3.2.8.1 Determination of relative crystallinity

The relative crystallinity of samples was quantitatively estimated following the method of Nara and Komiya (1983). A smooth curve which connected peak baselines was computer-plotted on the diffractogram. The area above the smooth curve was considered as the crystalline portion, and the lower area between the smooth curve and a linear baseline which connected the three points of intensity at 2θ of 4.5°, 6.6° and 35° was taken as the amorphous portion. The upper diffraction peak area and total diffraction area over the diffraction angle 4.5° - 35° 2θ were integrated by Origin software (version6.0, Microcal Inc., Northampton, MA, USA). The ratio of the upper area to the total diffraction area was calculated as the relative crystallinity (Fig. A-5 in Appendix).

3.2.8.2 Determination of B-polymorphic composition

B-polymorph content of samples was estimated by the method of Davydova *et al.*(1995). Different amounts (0 – 100%) of pure potato starch (B type) were thoroughly mixed with proportionate amounts (100 – 0%) of pure waxy corn (A type) starch. Moisture content was adjusted to ~19% as previously described. The diffractogram of each mixture was obtained (using the same diffractometer settings) and the ratio of peak area at 5.54° 2θ to the total peak area (crystalline portion) was calculated by Origin software (Version 6.0, Microcal. Inc). A standard curve was established by plotting the ratio versus the corresponding percentage of potato starch in the mixture (Fig. A-6 in Appendix).

3.2.9 Enzymatic hydrolysis by porcine pancreatic α-amylase

3.2.9.1 Hydrolysis pattern

Enzymatic digestibility studies on native starches were conducted using a crystalline suspension of porcine pancreatic α-amylase in 2.9M saturated sodium chloride containing 3mM calcium chloride (Sigma Chemical Co., St. Louis, MO, USA), in which the concentration of α-amylase was 32 mg/mL and the specific activity was 1370 units/mg protein. The procedure was essentially that of Knutson *et al.*(1982), however, a higher concentration of enzyme was used in this study (12 units/mg starch). Starch granules (0.2g, db) were suspended in distilled water (11mL) and then 9 mL of 0.1M phosphate buffer (pH 6.9) containing 0.006M NaCl were added. The slurry was pre-warmed for 30min at 37°C and gently stirred before adding 54.7 μL α-amylase suspension. The reaction mixtures were shaken manually on a daily basis to resuspend the deposited granules. One mL aliquots were withdrawn at specific time intervals, pipetted into 0.2 mL of 95% ethanol, and centrifuged (2000r.p.m.). Aliquots of the supernatant were analyzed for reducing sugar content (Bruner, 1964). The extent of hydrolysis was calculated as the percentage of initial starch converted to maltose. Controls without enzyme but subjected to the above experimental conditions were run concurrently. The experiment was performed in triplicate.

$$\text{Hydrolysis extent (\%)} = \frac{\text{Released reducing sugar as maltose (g)} \times 0.95}{\text{Initial starch weight (g)}} \times 100$$

3.2.9.2 Preparation of hydrolyzed residues

Residues obtained at various time intervals of hydrolysis were washed three times with distilled water, centrifuged (2000 r.p.m.) and freeze-dried.

3.2.9.3 Calculation of initial velocity

Initial velocity calculations for wrinkled pea and other legume starches were based on the data within the first 20min and 4h hydrolysis period, respectively. A linear regression line was plotted by the computer and the slope was regarded as the initial velocity (Fig. A-3 in Appendix).

3.2.10 Statistical analysis

All determinations were replicated three times, mean values and standard deviations were reported. Analysis of variance (ANOVA) was performed by Turkey's HSD test ($P<0.05$) using statistical software SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Chapter 4 Results and Discussion

4.1 Chemical composition of legume starches

Data on the composition of the legume starches are presented in Table 4-1. The purity of the starches was judged on the basis of composition and microscopic examination. The ash content ranged from 0.01 to 0.04%. This low value indicated that the starches were relatively free of hydrated fine fibers which are derived from the cell wall enclosing the starch granules. The nitrogen content was low in all starches (0.02-0.07%), indicating the absence of non-starch lipids (lipids associated with endosperm proteins). Therefore, the total lipid (obtained by acid hydrolysis) in the legume starches (0.35-0.84%) mainly represent the free and bound lipids. In all starches, the bound lipid content (0.26 – 0.81%) was higher than the surface lipid (0.01- 0.10%). Significant differences ($P<0.05$) in bound lipid content between cultivars was evident only in black bean [*Black Jack* (0.43%) > *CDC Nighthawk* (0.26%)] and pinto bean [*Othello* (0.57%) > *Sierra* (0.43%)] starches. The amounts of bound lipids in wrinkled pea (0.80%) and lentil (0.72 – 0.81%) were higher than those in the other legume starches (0.26 – 0.48%). In all starches, there was no significant difference ($P<0.05$) between the amount of lipid extracted by acid hydrolysis and that extracted by solvent extraction. Most of the data on the total lipid contents of legume starches reported in the literature have been obtained by the use of solvent systems that have been proven to be ineffective in removing bound lipids. Therefore, a meaningful comparison cannot be made.

The total amylose content of legume starches has generally been reported (Hoover and Sosulski, 1991) to be in the range of 24 – 65%. The total amylose content of the legume starches in this study (Table 4-1) ranged from 30.5 (lentil, *CDC Redwing*) to 78.4% (wrinkled pea). There was no significant difference ($P < 0.05$) in total amylose content between cultivars of the same species. The

Table 4-1

Chemical composition(%)[1] and some properties of legume starches

Characteristics	Black bean		Pinto bean		Wrinkled pea
	CDC Nighthawk	Black Jack	Othello	Sierra	
Yield (% of initial seeds)	16.37 ± 0.82^{e}	21.80 ± 1.06^{c,d}	28.25 ± 1.25^{b}	25.01 ± 1.50^{b,c}	21.60 ± 1.08^{c,d}
Moisture	10.99 ± 0.16^{b,c}	10.82 ± 0.10^{b,c}	11.38 ± 0.12^{b}	12.22 ± 0.20^{a}	11.76 ± 0.23^{a,b}
Ash	0.04 ± 0.01^{a}	0.03 ± 0.00^{a,b}	0.03 ± 0.01^{a,b}	0.02 ± 0.01^{a,b}	0.01 ± 0.00^{b}
Nitrogen	0.03 ± 0.01^{b,c}	0.05 ± 0.01^{a,b,c}	0.07 ± 0.02^{a,b}	0.08 ± 0.03^{a}	0.03 ± 0.01^{b,c}
Lipid					
Surface lipid[2]	0.10 ± 0.01^{a}	0.08 ± 0.01^{a,b}	0.06 ± 0.02^{b,c}	0.04 ± 0.01^{c,d,e}	0.05 ± 0.01^{b,c,d}
Bound lipid[3]	0.26 ± 0.02^{d}	0.43 ± 0.03^{c}	0.57 ± 0.03^{b}	0.43 ± 0.02^{c}	0.80 ± 0.05^{a}
Total lipid[4]	0.35 ± 0.02^{e}	0.52 ± 0.03^{d}	0.62 ± 0.04^{c}	0.48 ± 0.01^{d}	0.84 ± 0.02^{a}
Amylose content					
Apparent amylose[5]	35.21 ± 0.68^{b}	33.07 ± 1.19^{b,c}	28.36 ± 1.62^{d,e}	27.83 ± 0.81^{d,e}	68.84 ± 1.71^{a}
Total amylose[6]	39.32 ± 1.70^{b}	37.17 ± 0.68^{b,c}	31.93 ± 2.60^{d,e}	31.34 ± 0.36^{d,e}	78.42 ± 1.52^{a}
Lipid-complexed amylose[7]	10.37 ± 2.82^{a}	11.04 ± 3.14^{a}	11.18 ± 1.92^{a}	11.21 ± 1.06^{a}	12.22 ± 1.96^{a}
Starch damage	0.28 ± 0.03^{c,d}	0.27 ± 0.04^{c,d}	0.22 ± 0.03^{d,e}	0.24 ± 0.02^{c,d}	3.54 ± 0.02^{a}
Granule size (μm)					
Width	10.0 - 32.0	10.0 - 37.5	10.0 - 29.0	10.0 - 32.0	5.0 - 34.0
Length	10.0 - 40.0	10.0 - 41.0	10.0 - 40.0	10.0 - 42.0	5.0 - 37.0
Granule shape	round to oval	round to oval	round to oval	round to oval	irregular to compound /rounded rosette

1. Data with the same superscript in the same row are not significantly different ($P < 0.05$) by Tukey's HSD test. All data reported on dry basis and represent the mean ± SD of three determinations.
2. Lipids extracted by chloroform-methanol 2:1(v/v) at 25°C (mainly unbound lipids).
3. Lipids extracted by hot n-propanol-water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipids).
4. Lipids obtained by acid hydrolysis (24% HCl) of the native starch.
5. Apparent amylose determined by iodine binding without removal of free and bound lipids.
6. Total amylose determined by iodine binding after removal of free and bound lipids.
7. $\frac{\text{Total amylose - Apparent amylose}}{\text{Total amylose}} \times 100$

Table 4-1

Chemical composition(%)[1] and some properties of legume starches(cont'd)

Characteristics	Lentil		Smooth pea	
	CDC Robin	CDC Redwing	CDC Mozart	CDC Sonata
Yield(% of initial seeds)	27.44 ± 1.62^{b}	34.07 ± 2.04^{a}	19.40 ± 1.02^{d,e}	28.90 ± 2.20^{b}
Moisture	8.98 ± 0.32^{e}	9.87 ± 0.18^{d}	9.47 ± 0.15^{d,e}	10.47 ± 0.25^{c}
Ash	0.03 ± 0.01^{a,b}	0.03 ± 0.01^{a,b}	0.02 ± 0.01^{a,b}	0.02 ± 0.00^{a,b}
Nitrogen	0.04 ± 0.02^{a,b,c}	0.05 ± 0.01^{a,b,c}	0.08 ± 0.02^{a}	0.02 ± 0.01^{c}
Lipid				
Surface lipid[2]	0.01 ± 0.01^{e}	0.01 ± 0.01^{e}	0.02 ± 0.01^{d,e}	0.03 ± 0.01^{c,d,e}
Bound lipid[3]	0.81 ± 0.03^{a}	0.72 ± 0.05^{a}	0.47 ± 0.04^{b,c}	0.48 ± 0.03^{b,c}
Total lipid[4]	0.83 ± 0.02^{a}	0.71 ± 0.03^{b}	0.48 ± 0.03^{d}	0.52 ± 0.03^{d}
Amylose content				
Apparent amylose[5]	28.78 ± 1.29^{d,e}	27.35 ± 1.70^{e}	31.04 ± 0.21^{c,d}	30.63 ± 0.31^{c,d,e}
Total amylose[6]	32.29 ± 1.05^{d,e}	30.51 ± 0.63^{e}	35.09 ± 0.64^{c,d}	34.73 ± 1.09^{c,d}
Lipid-complexed amylose[7]	10.88 ± 0.90^{a}	10.34 ± 1.74^{a}	11.54 ± 1.29^{a}	11.83 ± 1.76^{a}
Starch damage	0.30 ± 0.01^{c}	0.15 ± 0.02^{e}	0.40 ± 0.03^{b}	0.43 ± 0.03^{b}
Granule size (μm)				
Width	8.0 - 28.0	6.0 - 27.0	8.0 - 32.0	9.0 - 34.0
Length	8.0 - 36.0	6.0 - 37.0	8.0 - 50.0	10.0 - 50.0
Shape	round to oval to irregular	round to oval to irregular	round to oval to irregular	oval to irregular

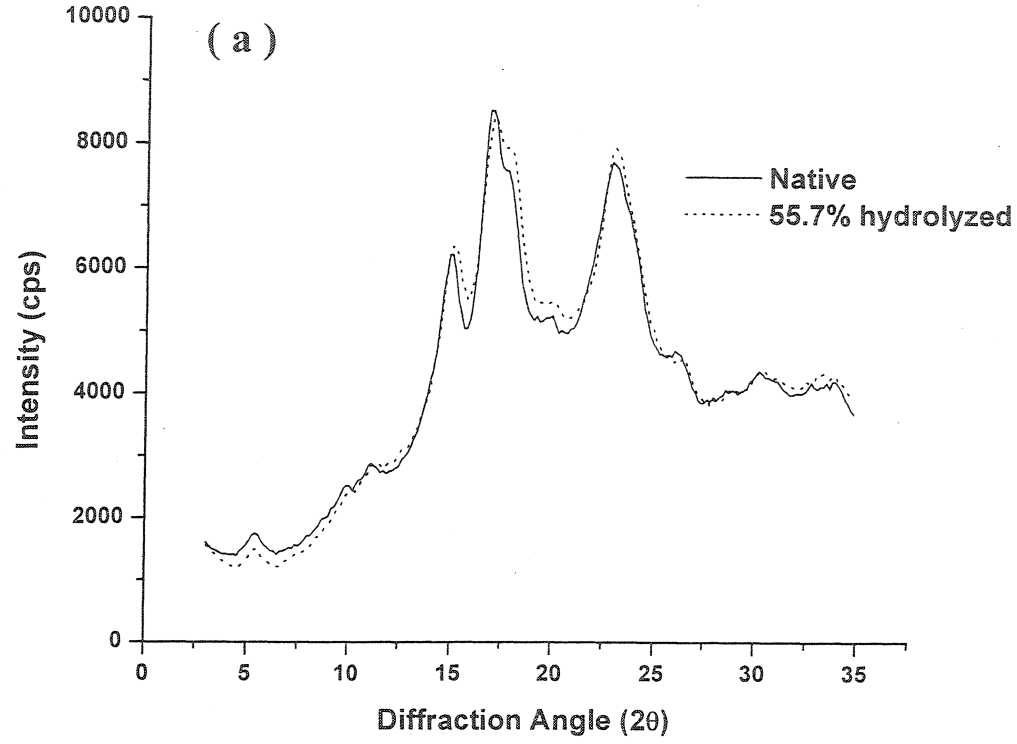
1. Data with the same superscript in the same row are not significantly different ($P < 0.05$) by Tukey's HSD test. All data reported on dry basis and represent the mean ± SD of three determinations.
2. Lipids extracted by chloroform-methanol 2:1(v/v) at 25°C (mainly unbound lipids).
3. Lipids extracted by hot n-propanol-water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipids).
4. Lipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids)
5. Apparent amylose determined by iodine binding without removal of free and bound lipids.
6. Total amylose determined by iodine binding after removal of free and bound lipids.
7. $\frac{\text{Total amylose - Apparent amylose}}{\text{Total amylose}} \times 100$

extent of granule damage in wrinkled pea starch (3.54%) was higher than that in the other legume starches (0.22 – 0.43%). Significant differences ($P < 0.05$) in starch damage between cultivars was observed only for lentil [*CDC Robin* (0.30) > *CDC Redwing* (0.15)].

4.2 X-ray diffraction of native starches

The X-ray diffraction pattern, relative crystallinity and 'B' polymorphic content of the legume starches are presented in Table 4-2 and Fig.4-1 (a, b) [the X-ray spectra of smooth pea, lentil and pinto bean starches (not shown) were similar to that of black bean starch (Fig. 4-1a)]. With the exception of wrinkled pea starch, all other starches showed the characteristic 'C' pattern of legume starches (Hoover and Sosulski, 1985a; Gernat *et al.*, 1990; Cheetham and Tao, 1998). The 'C' X-ray pattern was characterized by peaks at diffraction angles 2θ of 5.6°, 15°, 17°, 20°, and 23°. The X-ray spectrum of wrinkled pea (Fig. 4-1b) starch was of the 'B' type, representative of tuber starches, with prominent peaks at diffraction angles 2θ of 5.6°, 15°, 17°, 20°, 22°, and 23°. The intensity of the peak at 2θ=5.6° (characteristic of the 'B' polymorphic form) in wrinkled pea starch was higher than that in the other legume starches. However, the overall intensity of the peaks in wrinkled pea starch was much lower than that of the other legume starches (Fig. 4-1a). The relative crystallinity (RC) of wrinkled pea starch (17.7%) was much lower than that of the other legume starches (29.9 – 33.4%) (Table 4-2). There was no significant difference ($P < 0.05$) in RC either among or between cultivars of black bean, pinto bean, smooth pea and lentil (Table 4-2). The lower RC of wrinkled pea starch could be attributed to its low amylopectin content (Table 4-1). The 'B' polymorphic content of wrinkled pea starch (92.2%) was much higher than those of the other legume starches (27.1 – 37.5%) (Table 4-2). There was no significant difference ($P < 0.05$) in the 'B' polymorphic content (Table 4-2) between

Figure 4-1 X-ray diffraction patterns of native and hydrolyzed black bean (*Black Jack*) (Fig. 1a) and wrinkled pea starch (Fig. 1b). The X-ray pattern (native and hydrolyzed) of all other starches used in this study were similar to that of Fig. 1a.


(a)
10000
8000
6000
4000
2000
0
Intensity (cps)
0
5
10
15
20
25
30
35
Diffraction Angle (2θ)
Native
55.7% hydrolyzed

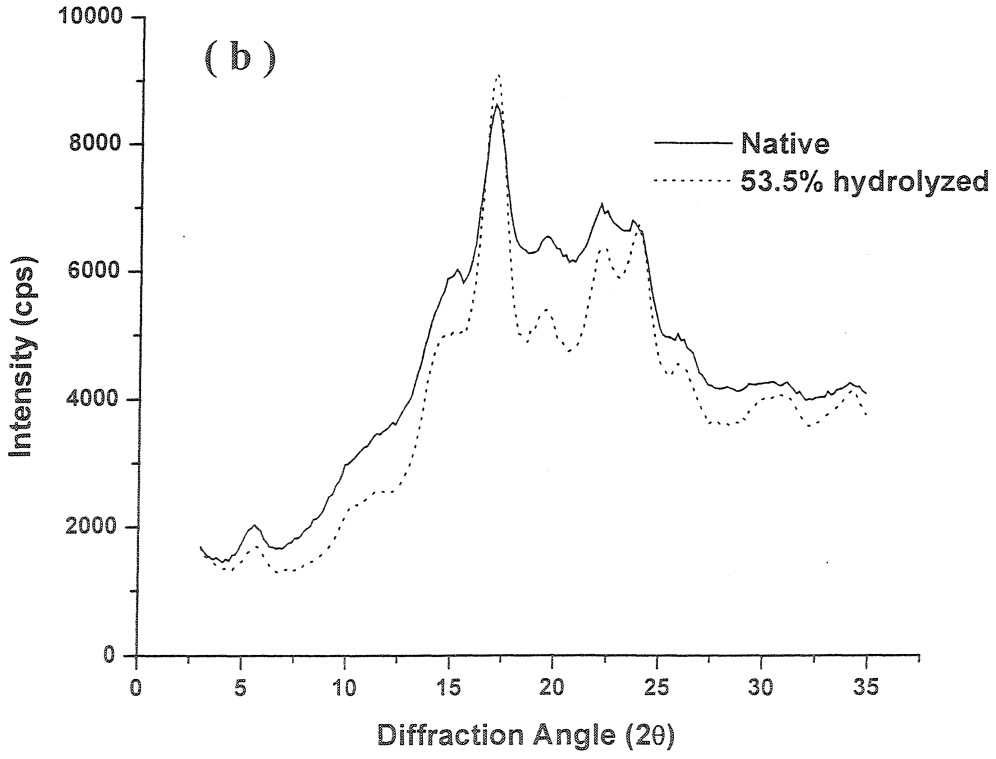

(b)
10000
8000
6000
4000
2000
0
Intensity (cps)
0
5
10
15
20
25
30
35
Diffraction Angle (2θ)
Native
53.5% hydrolyzed

Table 4-2

X-ray diffraction pattern, relative crystallinity and 'B' polymorphic content of legume starches[1]

Starch source & cultivar	Crystalline pattern	Relative crystallinity (%)[2]	'B' polymorphic content (%)[2]
Black bean			
CDC Nighthawk	C	32.1 ± 1.0^{a}	$32.1 \pm 2.4^{b,c}$
Black Jack	C	32.7 ± 2.2^{a}	$33.1 \pm 2.7^{b,c}$
Pinto bean	C		
Othello	C	33.4 ± 3.0^{a}	$32.1 \pm 2.0^{b,c}$
Sierra	C	33.0 ± 0.6^{a}	37.5 ± 2.1^{b}
Lentil	C		
CDC Robin	C	31.7 ± 2.5^{a}	28.1 ± 1.8^{c}
CDC Redwing	C	32.3 ± 2.2^{a}	36.1 ± 3.2^{b}
Smooth pea	C		
CDC Mozart	C	30.0 ± 2.0^{a}	27.1 ± 2.7^{c}
CDC Sonata	C	30.3 ± 2.4^{a}	28.8 ± 2.1^{c}
Wrinkled pea	B	17.7 ± 2.3^{b}	92.2 ± 3.0^{a}

1. Moisture content of all starches ~19.0%.
2. Mean ± SD of three determinations. Data with the same superscript within the same column are not significantly different ($P<0.05$).

cultivars of black bean, pinto bean and smooth pea. However, the 'B' polymorphic content of lentil cultivars differed significantly (P<0.05) [*CDC Redwing* (36.1%) > *CDC Robin* (28.1%)].

4.3 Swelling factor (SF) and amylose leaching (AML) at 80°C

The SF and AML of native legume starches are presented in Table 4-3. The SF ranged from 3.4 (wrinkled pea) to 18.4 (lentil- *CDC Robin*). No significant difference (P < 0.05) in SF was observed between cultivars of pinto bean, lentil, and smooth pea. However, cultivars of black bean differed in their SF [*Black Jack* (17.7) > *CDC Nighthawk* (8.2)]. The SF of black bean, lentil, pinto bean and smooth pea starches were generally lower than those reported for green pea (21.1), field pea (19.4), and mung bean (31.9), but comparable to those of beach pea (18.4) and grass pea (13.0) (Chavan *et al.*, 1999; Ratnayake *et al.*, 2001). The extent of AML at 80°C ranged from 11.0 (pinto bean – *Othello*) to 17.8% (smooth pea – *CDC Sonata*). There was a significant difference (P < 0.05) in AML between cultivars of black bean (*Black Jack* > *CDC Nighthawk*), pinto bean (*Sierra* > *Othello*) and lentil (*CDC Robin* > *CDC Redwing*). However, cultivars of smooth pea showed no significant difference (P<0.05) in AML. The extent of AML exhibited by the legume starches (Table 4-3) was comparable to that reported for beach pea (9.5%), grass pea (15.1%) and green pea (14.3%), but was lower than that reported for mung bean starch (Chavan *et al.*, 1999; Hoover *et al.*, 1997).

SF has been shown to be influenced by: 1) amylose-lipid complexes (Maningat and Juliano, 1980; Tester and Morrison, 1990; Tester *et al.*, 1993); 2) amylose content (Sasaki and Matsuki,, 1998); 3) extent of interaction between starch chains within the amorphous and crystalline domains of the granule (Hoover and Manuel, 1996) and 4) amylopectin molecular structure (Shi and Seib, 1992; Tester *et al.*, 1993; Sasaki and Matsuki, 1998). The differences in SF among legume starches and between cultivars of the same species (Table 4-3) could be attributed to the interplay of factors 2, 3,

Table 4-3
Swelling factor (SF) and amylose leaching (AML) of native legume starches at 80°C

Starch source & cultivar	SF[1]	AML(%)[1]
Black bean		
CDC Nighthawk	8.2 ± 1.9[b]	13.6 ± 0.5[b]
Black Jack	17.7 ± 0.4[a]	16.5 ± 0.6[a]
Pinto bean		
Othello	10.4 ± 0.9[b]	11.0 ± 0.4[b]
Sierra	9.9 ± 0.8[b]	13.0 ± 0.6[b]
Lentil		
CDC Robin	18.4 ± 0.9[a]	17.7 ± 0.9[a]
CDC Redwing	16.0 ± 1.0[a]	13.6 ± 0.3[b]
Smooth pea		
CDC Mozart	16.2 ± 1.3[a]	17.6 ± 0.5[a]
CDC Sonata	16.6 ± 0.5[a]	17.8 ± 0.2[a]
Wrinkled pea	3.4 ± 0.5[c]	11.1 ± 0.5[c]

1. Mean ± SD of three determinations. Data with the same superscript within the same column are not significantly different ($P<0.05$).

and 4, since there was no significant difference ($P < 0.05$) in the amount of lipid-complexed amylose chains (Table 4-1). The difference in SF between black bean cultivars (*Black Jack* > *CDC Nighthawk*) suggests the presence of longer amylopectin chains in *CDC Nighthawk.* Association between long amylopectin chains could result in the formation of a large number of double helices, and the helices could then form crystalline clusters which would increase granular stability, thereby reducing the extent of granular swelling. The lower SF (3.4) of wrinkled pea starch (Table 4-3) could be attributed to the interplay of the following factors: 1) lower amylopectin content (17.1%) (Table 4-1); 2) longer average amylopectin chain length (CL32 – 45 vs. CL24 – 27 for the other legume starches) (Colonna *et al.*, 1982; Colonna and Mercier, 1984; Biliaderis *et al.*, 1981; Hoover and Sosulski, 1991; Ratnayake *et al.*, 2002); and 3) closer packing of amylose chains [due to higher amylose (78.4%) content (Table 4-1)].

The extent of AML has been shown to be influenced by: 1) the extent of interaction between amylose chains (AM-AM) and/or between amylose and the outer branches of amylopectin (AM-AMP), and 2) the amount of lipid-complexed amylose chains (Ratnayake *et al.*, 2001; Hoover and Ratnayake, 2001). In this study, the extent of AML is mainly influenced by starch chain (AM-AM, AM-AMP) interactions within the native granule, since differences in the amount of lipid-complexed amylose chains between and among legume cultivars were not significant (Table 4-1). The results (Table 4-3) indicate that the extent of AM-AM and AM-AMP interactions between cultivars follows the trend: *CDC Nighthawk* > *Black Jack*; *Othello* > *Sierra*; *CDC Redwing* > *CDC Robin*; *CDC Mozart* ~ *CDC Sonata.* The results also indicate that the total amylose content *per se* does not influence AML, since wrinkled pea starch with its much higher amylose content (78.4%) exhibited nearly the same degree of AML as did pinto bean starch (32.0% amylose) (Table 4-1).

4.4 Gelatinization parameters

The gelatinization transition temperatures [T_o (onset), T_p (peak), T_c (conclusion) and ΔH (gelatinization enthalpy)] of native starches are presented in Table 4-4. T_o, T_p, T_c and $T_c - T_o$ of both cultivars of pinto bean were significantly ($P < 0.05$) higher than those of the other legume starches. There were significant ($P < 0.05$) differences in T_o, T_p and T_c between cultivars of black bean (*CDC Nighthawk* > *Black Jack*), pinto bean (*Othello* > *Sierra*) and lentil (*CDC Redwing* > *CDC Robin*). Wrinkled pea starch showed no endotherm (Table 4-4). Significant differences in ΔH were evident only between cultivars of black bean (*CDC nighthawk* > *black jack*) and lentil (*CDC redwing* > *CDC robin*). Noda *et al.* (1998) demonstrated that gelatinization temperatures are influenced by the molecular architecture of the crystalline region which corresponds to the distribution of amylopectin short chains (DP6 – 11) and not by the proportion of crystalline region, which corresponds to the amylose/amylopectin ratio. The above authors showed by studies in fifty one cultivars of sweet potato and twenty seven cultivars of buckwheat starches that a low T_o, T_p and T_c reflects the presence of abundant short amylopectin chains. Shi and Seib (1995) have also shown by studies on *ae wx*, *ae du wx*, *wx* and *du wx* maize starch, that *ae wx* starch having the lowest proportion of short chains (DP6 – 11) exhibited the highest gelatinization temperature and enthalpy. This suggests that the higher T_o, T_p and T_c shown by black bean and pinto bean starches indicate the presence of longer amylopectin chains (Table 4-4). The wider $T_c - T_o$ exhibited by pinto and black bean starches (Table 4-4) suggests the presence of crystallites of varying stability.

Waigh *et al.* (2000b) have postulated that two stages are involved during starch gelatinization in excess water. The first stage involves a slow side by side dissociation of helices and the second stage involves a rapid helix → coil transition. Cooke and Gidley (1992) have claimed that ΔH reflects

Table 4-4

Gelatinization characteristics of native legume starches[1]

Starch source & cultivar	To[2](°C)	Tp(°C)	Tc(°C)	Tc - To(°C)	ΔH / AP[3](mJ/mg)
Black bean					
Black Jack	61.0 ± 0.2^{d}	70.9 ± 0.3^{c}	81.2 ± 0.3^{d}	20.3 ± 0.5^{b}	17.8 ± 0.6^{b,c,d}
CDC Nighthawk	65.7 ± 0.3^{a}	74.9 ± 0.4^{b}	86.7 ± 0.2^{b}	21.0 ± 0.1^{b}	20.1 ± 1.0^{a}
Pinto bean					
Othello	64.5 ± 0.2^{b}	76.5 ± 0.6^{a}	88.8 ± 0.3^{a}	24.3 ± 0.4^{a}	17.9 ± 0.3^{b,c}
Sierra	63.3 ± 0.2^{c}	70.9 ± 0.2^{c}	85.1 ± 0.7^{c}	21.8 ± 0.5^{b}	18.8 ± 0.1^{a,b}
Smooth pea					
CDC Sonata	60.1 ± 0.2^{e}	66.0 ± 0.2^{e}	76.4 ± 0.2^{e}	16.3 ± 0.4^{c}	15.5 ± 0.5^{e,f}
CDC Mozart	60.0 ± 0.4^{e}	66.6 ± 0.1^{e}	77.5 ± 0.4^{e}	17.5 ± 0.7^{c}	16.6 ± 0.8^{c,d,e}
Lentil					
CDC Redwing	63.9 ± 0.1^{b,c}	70.6 ± 0.1^{c}	80.1 ± 0.9^{d}	16.2 ± 1.0^{c}	16.3 ± 0.4^{d,e}
CDC Robin	61.1 ± 0.2^{d}	67.7 ± 0.1^{d}	77.3 ± 0.3^{e}	16.2 ± 0.2^{c}	14.6 ± 0.1^{f}
Wrinkled pea[4]	—	—	—	—	—

1. Mean ± SD of three determinations. Data with the same superscript in the same column are not significantly different ($P < 0.05$).
2. T_o, T_p, T_c indicate the onset, peak and conclusion temperature of gelatinization, respectively.
3. Gelatinization enthalpy (mJ/mg) / Amylopectin content (%)
4. Gelatinization characteristics were not detected within the temperature range 25°C to 145°C.

primarily the loss of double helical order rather than loss of crystalline register. The larger ΔH values for starches of black bean and pinto bean cultivars (Table 4-4) suggest that interactions via hydrogen bonding between double helices that are packed in clusters forming the crystalline region of the above starches are probably more extensive (due to longer chains in amylopectin) than in smooth pea and lentil starches. Consequently, the ΔH associated with dissociation and unraveling (hydrogen bonds are broken during both stages of gelatinization) and melting of the double helices would be of a higher order of magnitude in pinto bean and black bean starches.

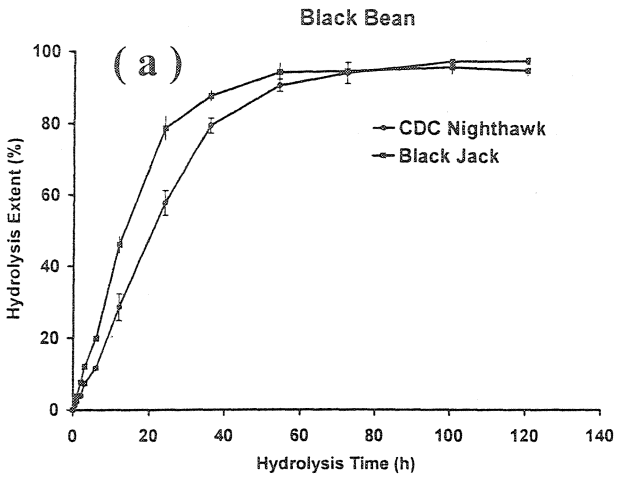
Jenkins (1994) has postulated that in excess water, gelatinization is primarily a swelling driven process. Water uptake by the amorphous background regions is accompanied by swelling within these regions. Swelling acts to destabilize the amylopectin crystallites within the crystalline lamellae, which are broken, Thus, the DSC endotherm represents solvation assisted melting of amylopectin crystallites. This suggests, that the absence of an endotherm for wrinkled pea starch (within the temperature range 20 - 145°C) is probably due to its low degree of swelling (Table 4-3) and consequently, a higher thermal input (> 145°C) would be required for crystallite melting.

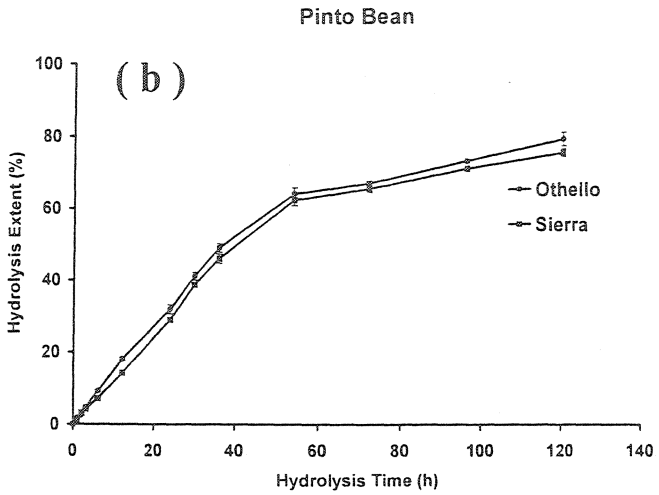
4.5 Hydrolysis patterns

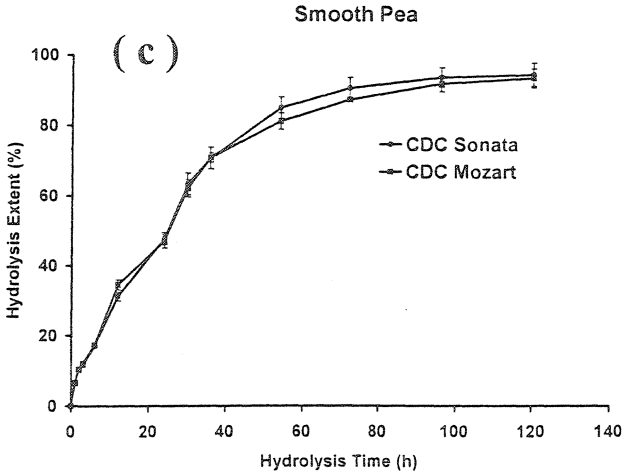
The hydrolysis by porcine pancreatic α-amylase in black bean (Fig. 4-2a), lentil (Fig. 4-2d) and wrinkled pea (Fig. 4-2e) starches was biphasic, a relatively rapid rate initially followed by a progressively decreasing rate thereafter (Fig. 4-2a,d,e). However, in pinto bean (Fig. 4-2b) and smooth pea (Fig. 4-2c) starches, the decrease in the rate of hydrolysis, following the initial rapid increase was much less than in the other starches (Fig. 4-2a,d,e). The hydrolysis curves of black bean cultivars (Fig. 4-2a), lentil cultivars (Fig. 4-2d), and wrinkled pea (Fig. 4-2e) showed a plateau at hydrolysis levels of 93 (Fig. 4-2a), 85 (Fig. 4-2d) and 65% (Fig. 4-2e), respectively.

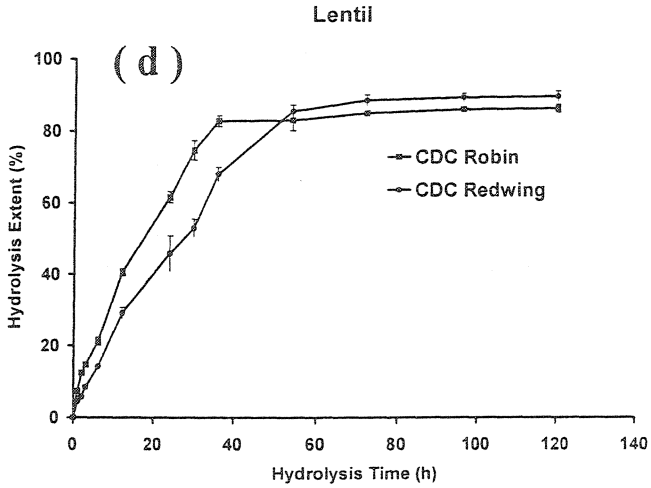
Figure 4-2 Hydrolysis patterns (37°C) by porcine pancreatic α-amylase with legume starches.

a) black bean; b) pinto bean; c) smooth pea; d) lentil; e) wrinkled pea.


Black Bean
(a)
CDC Nighthawk
Black Jack
Hydrolysis Extent (%)
Hydrolysis Time (h)


Pinto Bean
(b)
Othello
Sierra
Hydrolysis Extent (%)
Hydrolysis Time (h)


Smooth Pea
(c)
CDC Sonata
CDC Mozart
Hydrolysis Extent (%)
Hydrolysis Time (h)


Lentil
(d)
CDC Robin
CDC Redwing
Hydrolysis Extent (%)
Hydrolysis Time (h)

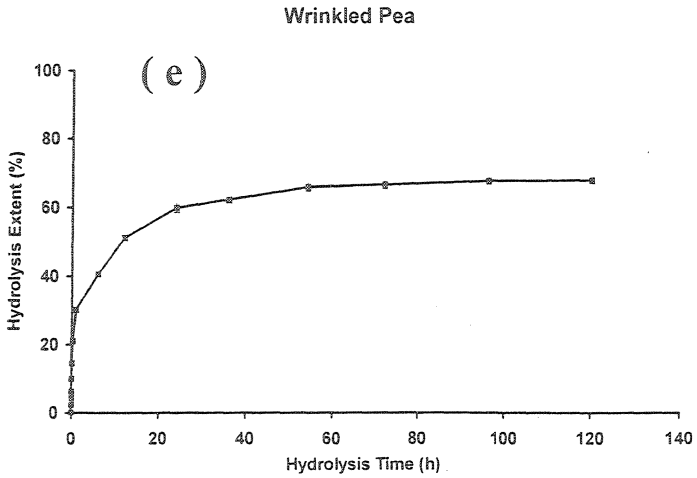

Wrinkled Pea
(e)
Hydrolysis Extent (%)
Hydrolysis Time (h)

Table 4-5

Initial velocity of a-amylase hydrolysis of legume starches[1]

Starch source & cultivar	Initial velocity[2] (% / h)
Black bean	
Black Jack	3.9 ± 0.3[c]
CDC Nighthawk	2.3 ± 0.4[d,e]
Pinto bean	
Othello	1.5 ± 0.3[e]
Sierra	1.5 ± 0.2[e]
Smooth pea	
CDC Sonata	5.5 ± 0.4[b]
CDC Mozart	5.4 ± 0.5[b]
Lentil	
CDC Robin	5.4 ± 0.2[b]
CDC Redwing	2.9 ± 0.3[d]
Wrinkled pea	241.6 ± 5.6[a]

1. Initial velocity calculation for wrinkled pea and the other legume starches are based on the data within the first 20min and 4h, respectively. Data represent mean ± SD of three determinations.
2. Data with the same superscript in the column are not significantly different ($P<0.05$).

This plateau, appeared at the same time for black bean cultivars (55 h), but was different for cultivars of lentil [*CDC Robin* (35h), *CDC Redwing* (55 h)]. Cultivars of pinto bean (Fig.4-2b) and smooth pea (Fig.4-2c) starches did not exhibit a plateau during the hydrolysis period. Wrinkled pea starch exhibited a higher initial velocity (241.6%/h) than the other legume starches (1.4 - 5.5%/h) (Table 4-5). Difference in initial velocity between cultivars was evident only in black bean [*Black Jack* (3.9%/h) > *CDC Nighthawk* (2.3%/h)] and lentil [*CDC Robin* (5.4%/h) > *CDC Redwing* (2.9%/h)]. During the initial rapid phase of hydrolysis, cultivars of black bean (Fig. 4-2a) and lentil (Fig. 4-2d) were hydrolyzed to different extents. This difference was most marked between the 5^{th} and 40^{th} hour of hydrolysis in both black bean (*Black Jack* > *CDC Nighthawk*) and lentil (*CDC Robin* > *CDC Redwing*) cultivars. However, during the above time period, there was no significant difference ($P<0.05$) in the extent of hydrolysis between cultivars of pinto bean (Fig. 4-2b) and smooth pea (Fig. 4-2c) starches. After 120h, there was no difference in the extent of hydrolysis between cultivars of each legume species (Fig. 4-2). At the end of this time period, the extent of hydrolysis among the legume starches followed the order: black bean > lentil > smooth pea > wrinkled pea > pinto bean (Fig. 4-2).

It is appropriate at this stage to give a brief description of the mechanism of α-amylase action, which would then enable a subsequent discussion of the hydrolysis kinetics of the legume starches.

Porcine pancreatic α-amylase (PPA) has been shown to have five binding sites with the catalytic site located between subsites 2 and 3, with two subsites to the right and three subsites to the left of the catalytic site (Robyt and French, 1970). These authors have shown that only the chain to the right diffuses away after the initial cleavage and the remaining chain to the left diffuses to fill the open binding subsites to give maltose (G2), maltotriose (G3) and maltotetraose (G4) as products in a multiple attack mechanism. The products of hydrolysis, particularly G2 and G3, are known to have an inhibitory effect on the action of α-amylase *in vitro* (Robyt and French, 1970; Elodi *et al.*, 1972;

Leloup *et al.*, 1991). G2 and G3 have been shown to bind strongly to PPA, thereby impeding their adsorption onto crystalline spherulites of short chain amylose (Leloup *et al.*, 1991).

The appearance of a plateau during hydrolysis of black bean (Fig. 4-2a), smooth pea (Fig. 4-2c), lentil (Fig. 4-2d) and wrinkled pea (Fig. 4-2e) starches reflects the interplay of the following factors: 1) inhibition of α-amylase activity by G2 and G3 (the occupation of the subsites to the left of the catalytic center by G2 and G3 would prevent further hydrolysis of starch chains); 2) formation of crystalline regions during hydrolysis (hydrolyzed amylose chains may retrograde forming crystalline regions which could hinder the accessibility of α-amylase to the glucosidic bond), and 3) depletion of substrate. The absence of a plateau in pinto bean starch (Fig. 4-2b), even after 120h of hydrolysis, suggests strong interactions between starch chains within the amorphous and crystalline domains of the native granule. These interactions probably reduce the degree of accessibility of the glucosidic bonds to α-amylase, thereby decreasing the rate of release of G2 and G3 during hydrolysis. Thus, the time taken for α-amylase inhibition by G2 and G3 would be much longer in pinto bean than in other legume starches.

Jenkins and Donald (1995) have postulated that co-crystallization of amylose with amylopectin disrupts amylopectin crystallites. Their postulation was based on the observation that the electron density difference between the crystalline and amorphous lamella decreases with increased amylose content. Cheetham and Tao (1998) have shown by X-ray diffraction studies on native maize starches of varying amylose content (0 – 84%) that crystallinity decreases with increased amylose content in both 'A' and 'C' type starches. This suggests that the low RC (17.7%) of wrinkled pea starch (Table 4-2) is probably due to disrupted amylopectin crystallites. The extent of this disruption is likely to be higher in wrinkled pea starch than in the other legume starches, due to its higher amylose content (78.1%) (Table 4-1) and the longer average amylopectin chain length [$\overline{CL}$ 32 - 45 vs. $\overline{CL}$ 24 – 27] in the other legume

starches (Biliaderis *et al.*, 1981; Colonna *et al.*, 1982; Colonna and Mercier, 1984; Hoover and Sosulski, 1991; Ratnayake *et al.*, 2002)]. Thus, the higher initial velocity (Table 4-5) exhibited by wrinkled pea starch could be a reflection of a highly disrupted crystalline structure. It is also likely, that the higher initial velocity of wrinkled pea starch could also be a reflection of the higher extent of granule damage incurred during starch isolation (Table 4-1).

Tester and Sommerville (2000) have postulated that granular swelling is controlled by granule order which controls α-amylolysis. The difference between black bean cultivars with respect to SF (*Black Jack* > *CDC Nighthawk*) and AML (*Black Jack* > *CDC Nighthawk*) at 80°C (Table 4-3) suggests that starch chain interactions (amylose-amylose, amylopectin-amylopectin, amylose-amylopecin) within native starch granules are of a higher order of magnitude in *CDC Nighthawk.* Strong interactions between starch chains would not only reduce granular swelling at 37°C (assay temperature), but also could hinder the chair → half chair conformational transition (the degree of accessibility of the glucosidic oxygen to α-amylase is influenced by this transition) of the D-glucopyranosyl unit during hydrolysis [Hoover, 2000]. This would then explain the initial velocity difference between the black bean cultivars (*Black Jack* > *CDC Nighthawk*) (Table 4-5). The difference in initial velocity between cultivars of lentil (*CDC Robin* > *CDC Redwing*) can be attributed to a lower degree of interaction between starch chains in *CDC Robin* [indicated by a higher SF and a higher degree of AML (Table 4-3)]. The marginal difference in SF and AML between cultivars of pinto bean and smooth pea (Table 4-3) may explain their nearly identical initial velocities (Table 4-5).

The above explanation based on SF and AML seems plausible, since differences between cultivars with respect to granule size (Table 4-1), amylose content (Table 4-1), starch damage (Table 4-1), lipid-complexed amylose chains (Table 4-1), relative crystallinity (Table 4-2) and 'B' polymorph

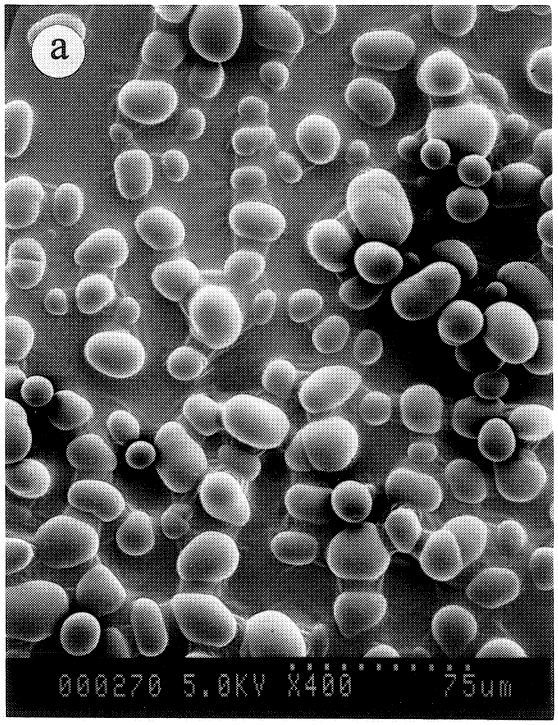
content (Table 4-2) are too small to account for the large difference in initial velocity between cultivars of black bean and lentil.

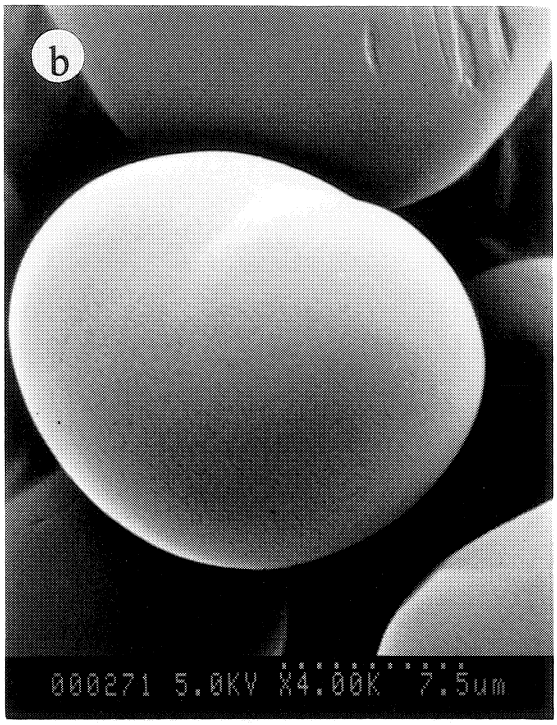
4.6 Morphology of native starch granules and enzyme hydrolyzed starch residues

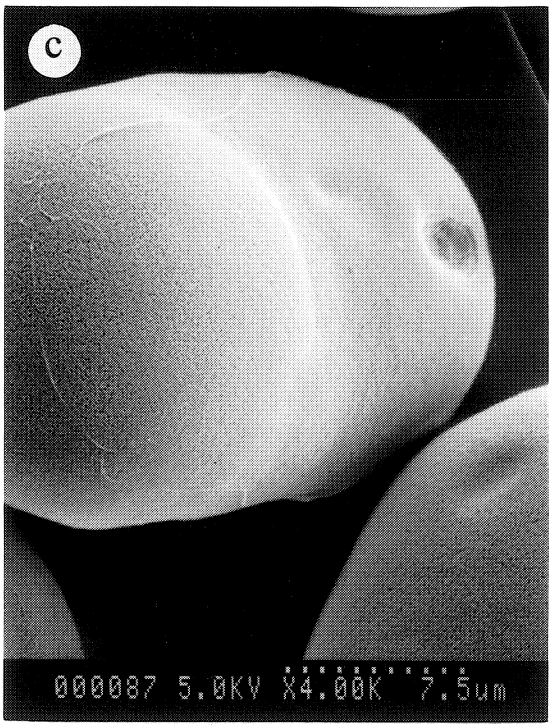
The morphologies of native legume starches and their hydrolyzed residues (at nearly equivalent levels of hydrolysis) are presented in Figs. 4-3 – 4-7. The granules of native black bean (Figs. 4-3a, b), pinto bean (Figs. 4-4a, b), smooth pea (Figs. 4-5a, b) and lentil (Figs. 4-6a, b) ranged from oval to irregular in shape. The width and length of the granules were within the range 5.0 – 37.5μm and 5.0 - 50μm, respectively (Table 4-1). Wrinkled pea starch appeared to be a mixture of simple and compound granules (Figs. 4-7a,b,c). Many of the compound granules contained clusters (3–5) of individual granules. Many of the simple granules (mainly small granules) were round in shape; whereas large granules (forming the cluster) were irregular in shape. The width and length of small and large granules ranged from 5.0 to 34.0μm and 5.0 to 37.0μm, respectively (Table 4-1). In native wrinkled pea starch, some of the larger granules showed extensive damage, resulting in splitting and exposure of the internal layering (Fig. 4-7b). A similar observation was reported by Bertoft *et al* (1993). The granule surfaces of native pinto bean (Figs. 4-4a, b), smooth pea (Figs. 4-5a, b) and lentil (Figs. 4-6a, b) starches were smooth and showed no evidence of pores, fissures or indentations. However, in black bean (Figs. 4-3a, b) and wrinkled pea (Figs. 4-7a,b,c) starches, indentations were present on the surface of some granules, whereas others were smooth and free of pores, fissures and indentations. There was no difference in granule morphology between cultivars of the same legume species.

The mode of α-amylase attack was examined by SEM during the early stages of hydrolysis (< 20%), and at nearly equivalent levels of hydrolysis. Hydrolyzed (15.4%) black bean starch (*Black*

Figure 4-3 Scanning electron micrographs of native black bean (*Black Jack*) granules (a & b) and hydrolyzed (15.4%) granules (c & d).


a
000270 5.0KV X400 75um


b
000271 5.0KV X4.00K 7.5um


c
000087 5.0KV X4.00K 7.5um

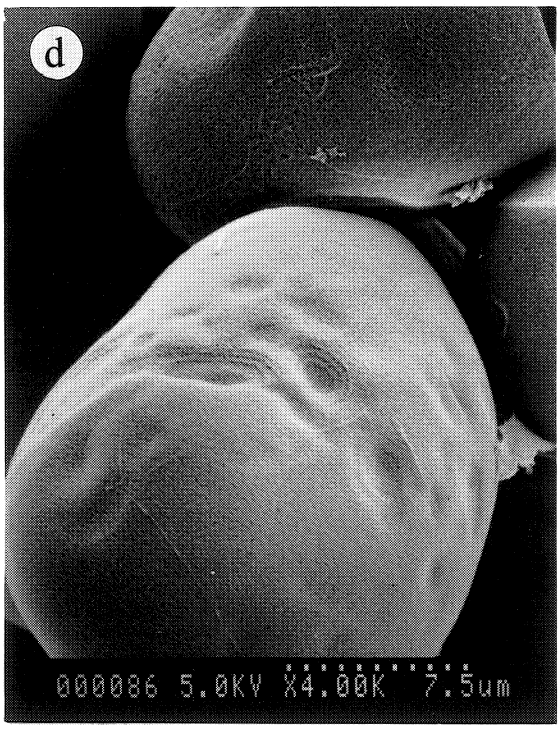
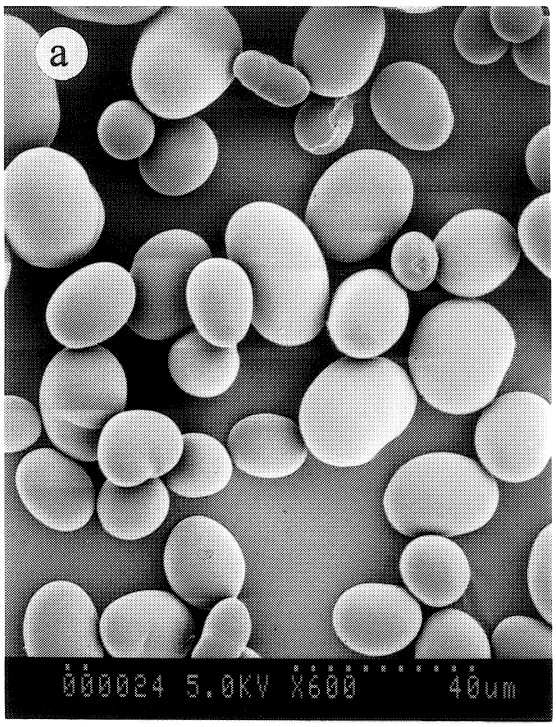
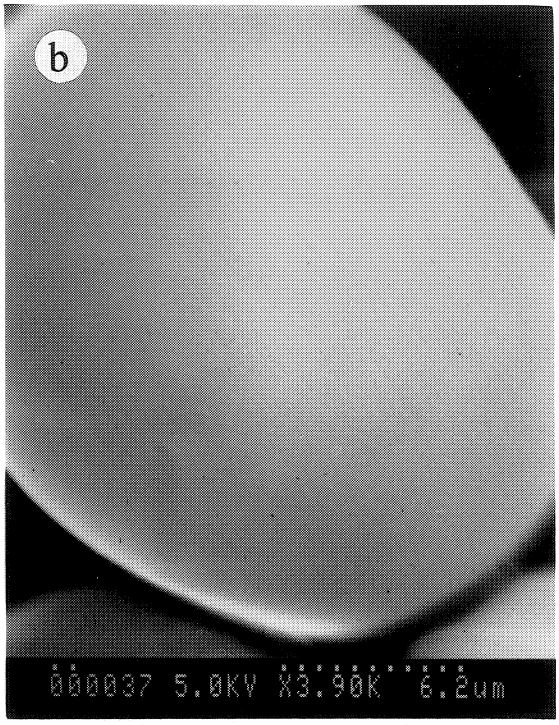
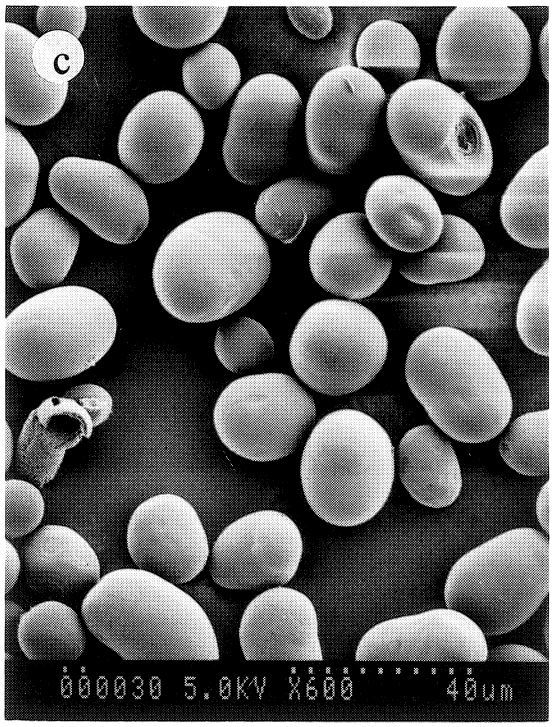

d
000086 5.0KV X4.00K 7.5um

Figure 4-4 Scanning electron micrographs of native pinto bean (*Othello*) granules (a & b) and hydrolyzed (18.1%) granules (c & d).


a
000024 5.0KV X600 40um


b
000037 5.0KV X3.90K 6.2um


c
000030 5.0KV X600 40um

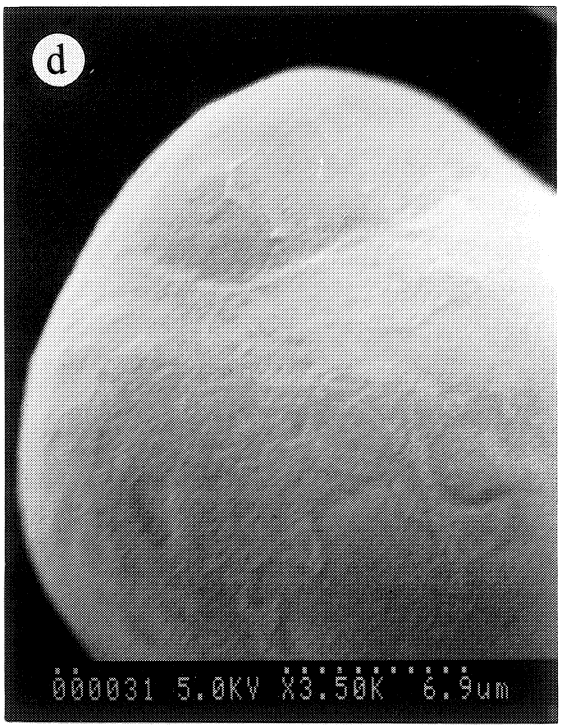
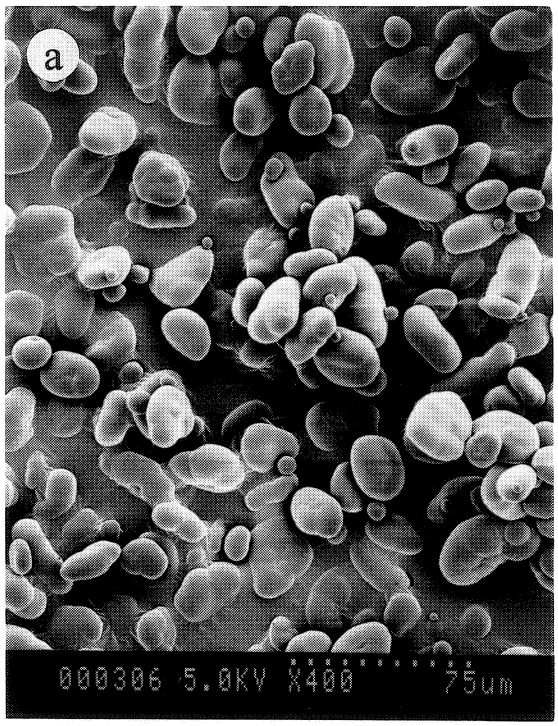
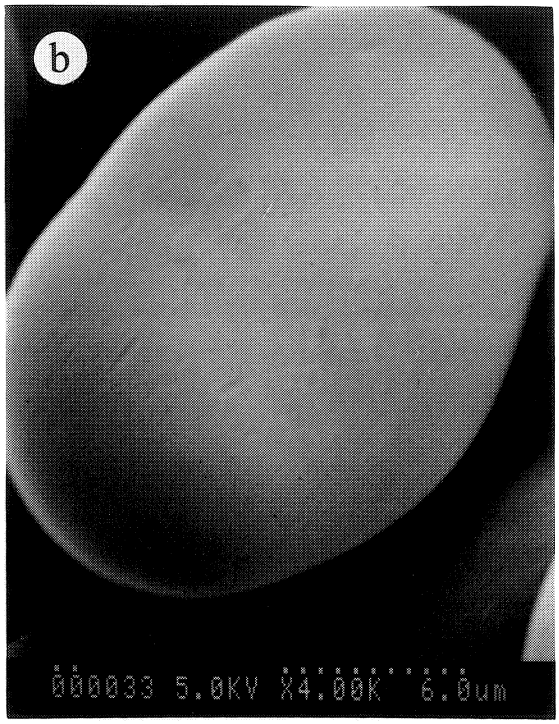
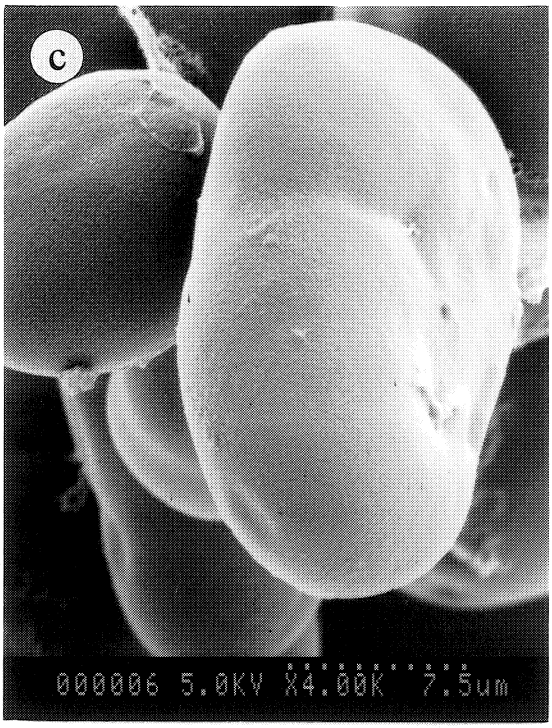

d
000031 5.0KV X3.50K 6.9um

Figure 4-5 Scanning electron micrographs of native smooth pea (*CDC Sonata*) granules (a & b) and hydrolyzed (17.0%) granules (c & d).


a
000306 5.0KV X400 75um


b
000033 5.0KV X4.00K 6.0um


c
000006 5.0KV X4.00K 7.5um

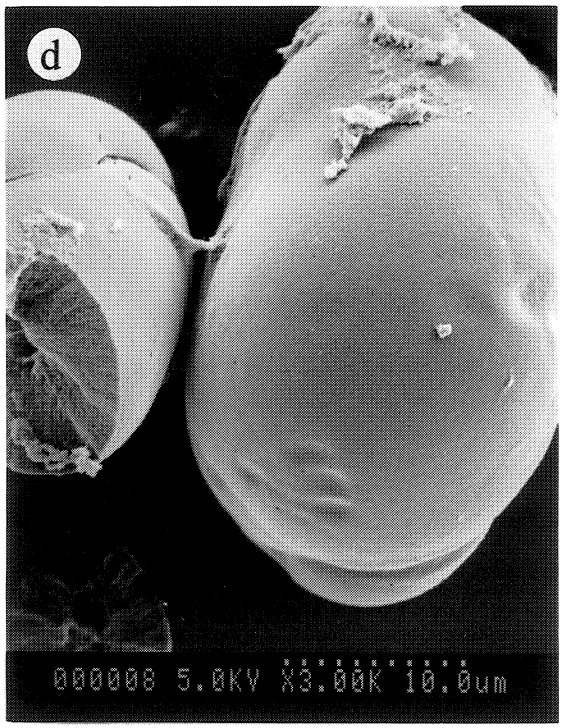
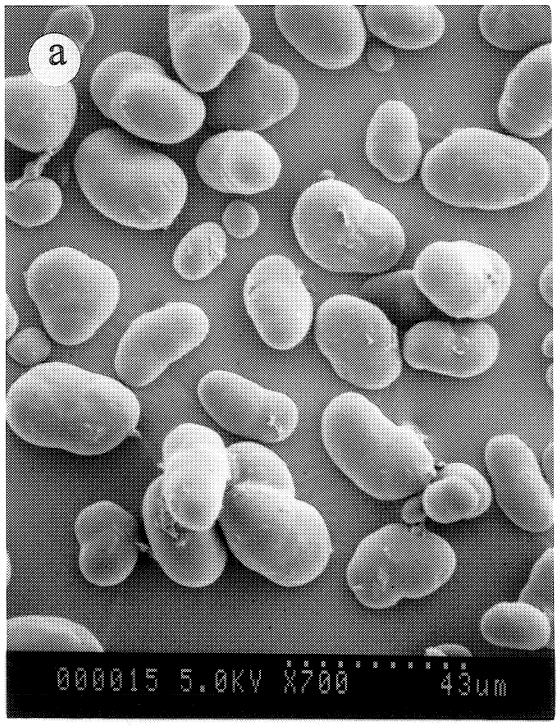
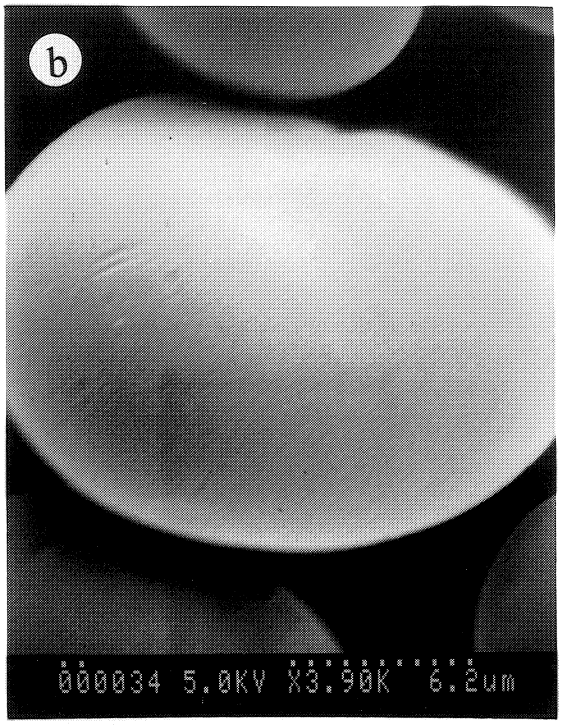
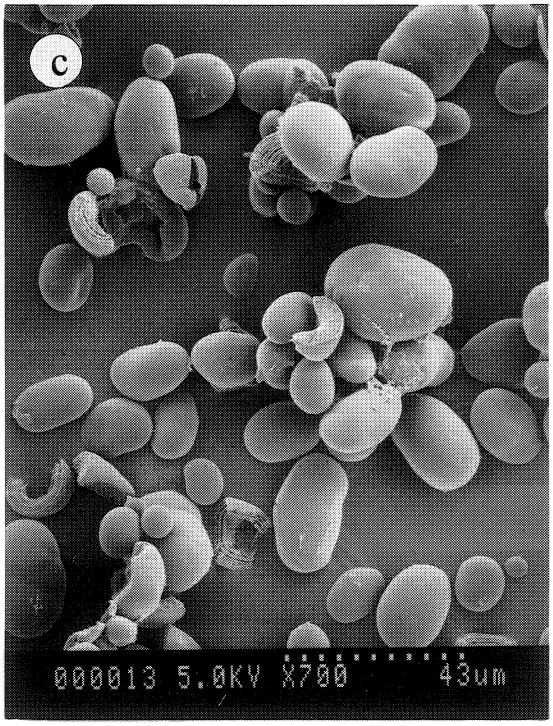

d
000008 5.0KV X3.00K 10.0um

Figure 4-6 Scanning electron micrographs of native lentil (*CDC Redwing*) granules (a & b) and hydrolyzed (14.2%) granules (c & d).


a
000015 5.0KV X700 43um


b
000034 5.0KV X3.90K 6.2um


c
000013 5.0KV X700 43um

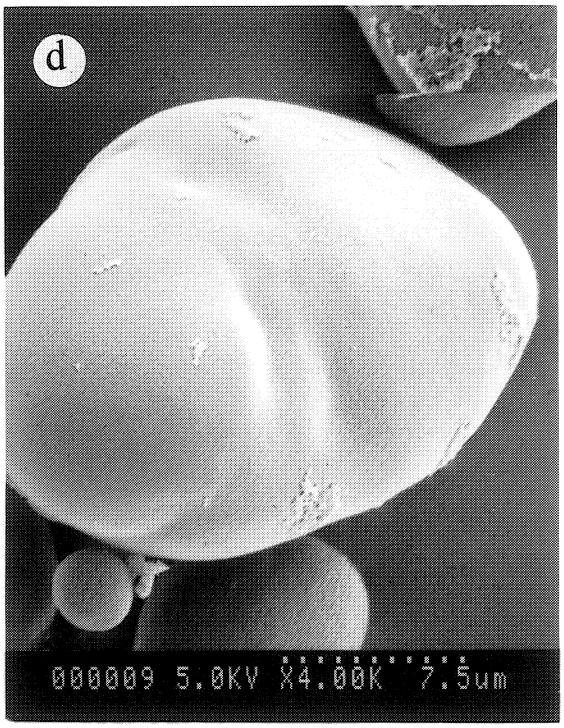
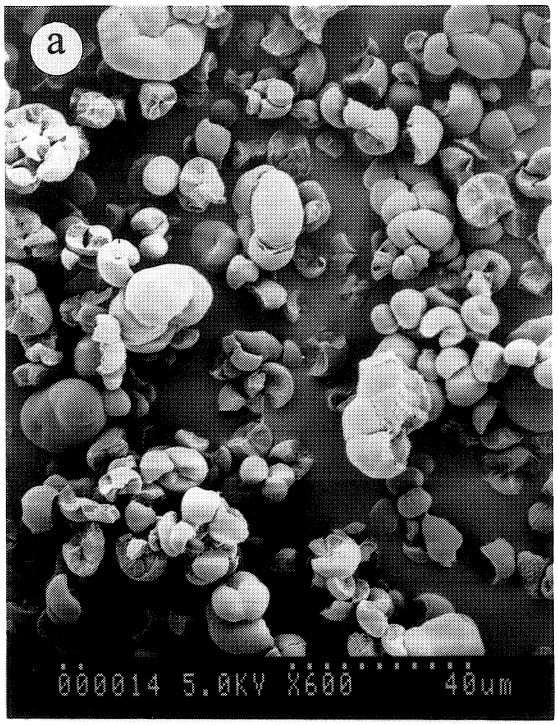
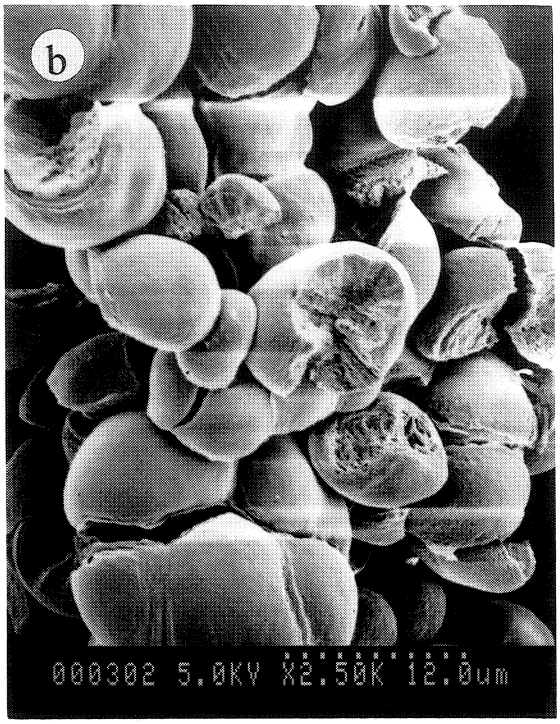
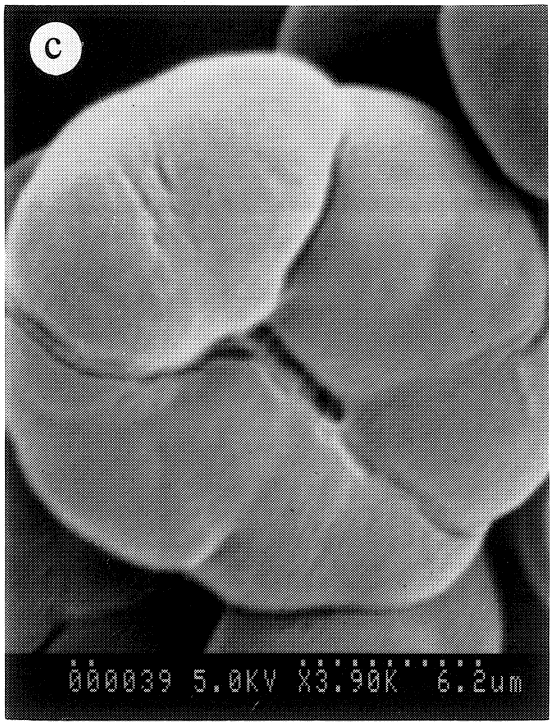
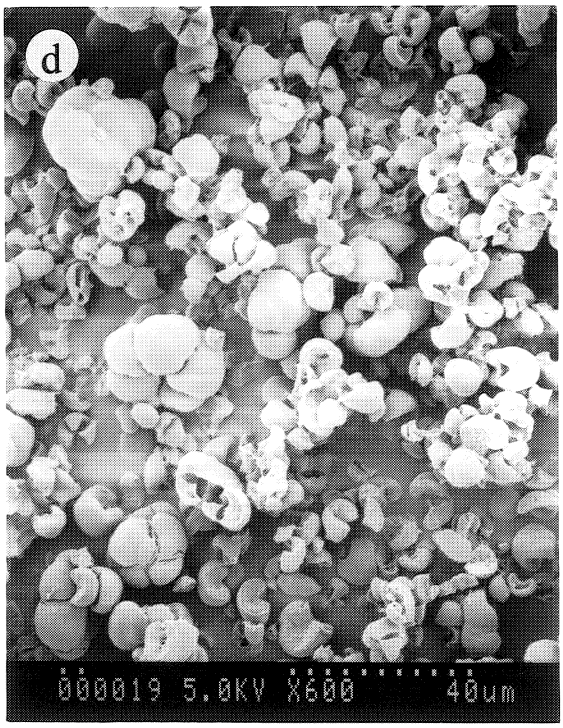

d
000009 5.0KV X4.00K 7.5um

Figure 4-7 Scanning electron micrographs of native (a, b, c) wrinkled pea granules and hydrolyzed (16.2%) granules (d).


a
000014 5.0KV X600 40um


b
000302 5.0KV X2.50K 12.0um


c
000039 5.0KV X3.90K 6.2um


d
000019 5.0KV X600 40um

Jack) granules showed slightly roughened surfaces and disc like depressions and the number of depressions varied from granule to granule (Figs. 4-3c, d). Roughened surfaces and disc like depressions were also visible on the surfaces of hydrolyzed (18.1%) pinto bean (*Othello*) starch. However, the depth of these depressions (Fig. 4-4d) was much lower than that in black bean (Fig. 4-3d) starch. In hydrolyzed (17%) smooth pea starch (*CDC Sonata*), some granules (~ <1% of the total population) had fragmented so that their interior parts were exposed (Fig. 4-5d), whereas, the major population of the granules was intact and exhibited only roughened surfaces and disc like depressions (Figs. 4-5c,d). Lentil starch (*CDC Robin*) at 14.2% hydrolysis behaved similarly to smooth pea starch with respect to roughened surfaces and granule fragmentation (< 1% of the granule population). However, none of the hydrolyzed granules showed disc like depressions on their surfaces. In hydrolyzed (16.2%) wrinkled pea starch (Fig. 4-7d), several of the large granules had fragmented, exposing their interior structure. However, some granules were still intact with no evidence of α-amylase attack. Furthermore, the extent of fragmentation in wrinkled pea starch was much higher than that in smooth pea and lentil starches. A similar pattern of hydrolysis was observed by Bertoft *et al.* (1993) on wrinkled pea starch hydrolyzed by *Bacillus amyloliquefaciens*.

4.7 X-ray analysis of hydrolyzed starch residues

The X-ray patterns of native and control (treated without α–amylase) and the hydrolyzed residues of the legume starches are presented in Figs. 4-1a, b and Table 4-6. There was no significant difference in the X-ray pattern, relative crystallinity or 'B' polymorphic content between native and control starches (Table 4-6). Hydrolysis did not change the X-ray pattern, relative crystallinity or the 'B' polymorphic content of black bean (*Black Jack*), pinto bean (*Othello*), smooth pea (*CDC Sonata*), and lentil (*CDC Robin*) starches (Table 4-6). This was also true for the other cultivars of the

Table 4-6
X-ray diffraction parameters of α-amylase hydrolyzed legume starch residues

Starch source & cultivar	Crystalline pattern	Relative crystallinity(%)[1]	'B' Polymorphic content(%)[1]
Black bean			
Black Jack			
native	C	32.7 ± 2.2[a]	33.1 ± 2.7[f]
control[2]	C	32.3 ± 2.2[a]	33.8 ± 2.8[f]
hydrolyzed (55.7%)	C	28.7 ± 2.5[a]	31.7 ± 2.0[f]
Pinto bean			
Othello			
native	C	33.4 ± 3.0[b]	32.1 ± 2.0[g]
control[2]	C	32.9 ± 2.6[b]	32.4 ± 1.2[g]
hydrolyzed (45.6%)	C	29.4 ± 2.0[b]	33.4 ± 1.9[g]
Smooth pea			
CDC Sonata			
native	C	30.3 ± 2.4[c]	28.8 ± 2.1[h]
control[2]	C	30.0 ± 1.7[c]	28.0 ± 1.7[h]
hydrolyzed (41.6%)	C	29.0 ± 1.7[c]	27.1 ± 2.6[h]
Lentil			
CDC Robin			
native	C	31.7 ± 2.5[d]	28.1 ± 1.8[i]
control[2]	C	31.0 ± 2.0[d]	28.1 ± 2.1[i]
hydrolyzed (48.9%)	C	28.2 ± 2.1[d]	27.8 ± 2.8[i]
Wrinkled pea			
native	B	17.7 ± 2.3[e]	92.2 ± 3.0[j]
control[2]	B	17.8 ± 1.7[e]	91.3 ± 2.3[j]
hydrolyzed (53.5%)	B	33.4 ± 3.1[e]	89.8 ± 2.2[j]

1. Mean ± SD. For each cultivar, data with the same superscript in the same column are not significantly different($P<0.05$).
2. Treated without α-amylase but subjected to the same experimental conditions.

above starches (data and figures not shown). The hydrolyzed residues of wrinkled pea starch also showed an unchanged X-ray pattern (Fig. 4-1b) and 'B' polymorphic content (Table 4-6). However, relative crystallinity increased (17.8 to 33.4%) substantially on hydrolysis (Table 4-6).

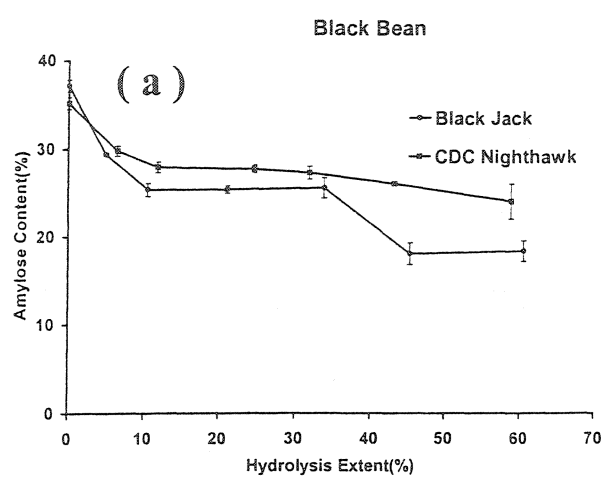
Several researchers have shown that α-amylases can simultaneously solubilize both amorphous and crystalline regions of starch granules (Leach and Schoch, 1961; Colonna *et al.*, 1988; Lauro *et al.*, 1999). This was based on the observation that α-amylolysis did not produce an increase in crystallline. However, crystallinity and gelatinization enthalpy of barley starches have been shown to decrease during the later stages of α-amylolysis (Lauro *et al.*, 1999). This suggests that extensive hydrolysis effectively destroys and solubilizes the crystallline areas of the granule. However, the exact mechanism by which starch crystallites are degraded by α-amylase remains controversial. Comparison of our X-ray data with that of Lauro *et al.*, 1999 suggests that crystallites of legume starches are more resistant to α-amylolysis than the 'A' type crystallites of barley starch. This is based on the observation that even at 55% hydrolysis, the relative crystallinity of black bean starch remained unchanged (Table 4-6). The increase in relative crystallinity on hydrolysis of wrinkled pea starch could be attributed to interaction (retrogradation) between partially hydrolyzed amylose chains (dextrins) during the hydrolysis period. This interaction would be of a greater order of magnitude in wrinkled pea starch, due to its higher amylose content (Table 4-1). A high amylose content may have resulted in amylose chains being in close proximity to each other within the amorphous domains of the granule, thus facilitating rapid interaction between hydrolyzed amylose chains. It is highly unlikely that the above interactions are influenced by the temperature of incubation (37°C), since the relative crystallinity of both native and control wrinkled pea starches were identical (Table 4-6).

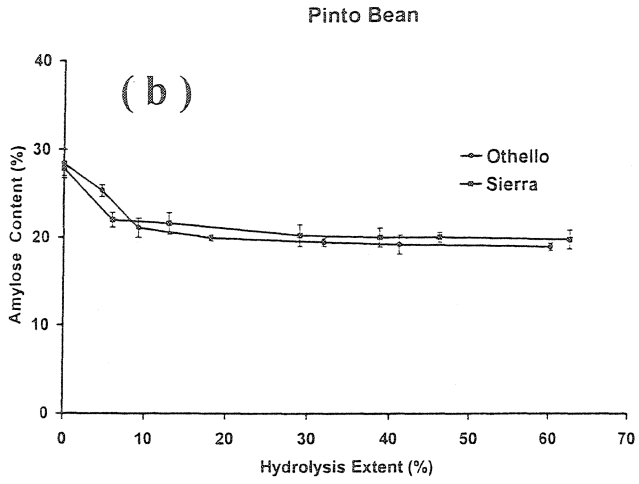
4.8 Apparent amylose content of hydrolyzed starch residues

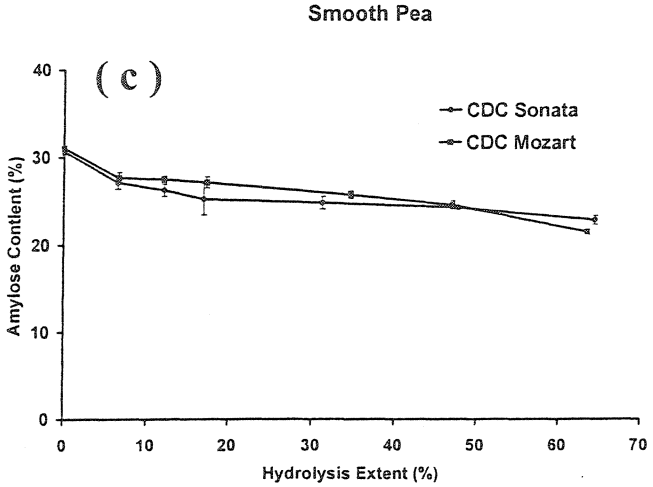
The changes in apparent amylose content (AAC) at different times during hydrolysis are presented in Fig. 4-8. In all starches, AAC decreased with hydrolysis time and the extent of this decrease was most pronounced in wrinkled pea starch. At 60% hydrolysis, AAC decreased from 68.5 to 28.0% for wrinkled pea starch. For the other legume starches, the decrease in AAC at 60% hydrolysis ranged from 9 to 21% (Fig. 4-8). There was no significant difference ($P<0.05$) in the extent of decrease in AAC between cultivars of pinto bean, smooth pea and lentil starches. However, in black bean starches, the decrease in AAC was higher in *Black Jack* (21%) than in *CDC Nighthawk* (12.0%).

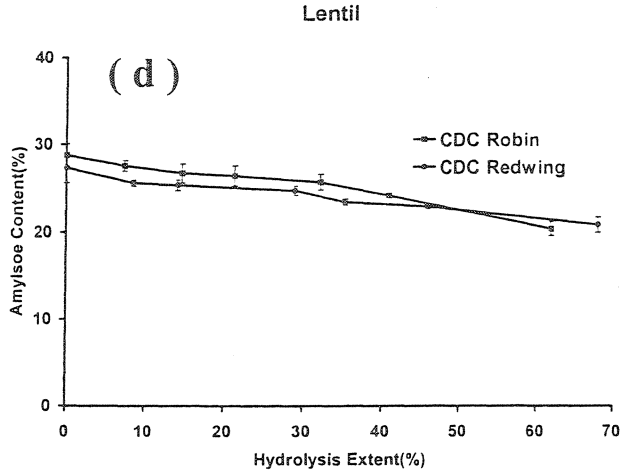
As discussed earlier, disruption of amylopectin crystallites by amylose was most pronounced in wrinkled pea starch. Consequently, the degree of accessibility of α-amylase to amylose chains within the amorphous domains of the granule would be of a very high order of magnitude in wrinkled pea starch. This would explain the rapid and large decrease in AAC content upon hydrolysis (Fig. 4-8e). Amylose leaching measurements (Table 4-3) showed that among legume starches, differences in the magnitude of starch chain interactions (AM-AM and/or AM-AMP) were more pronounced between cultivars of black bean (*CDC Nighthawk* > *Black Jack*). This suggests that the differences in the extent of decrease in AAC between black bean cultivars (Fig. 4-8a) reflect differences in the degree of accessibility of α-amylase to amylose chains within the amorphous domains of the granule. Biliaderis *et al.* (1981) showed that on acid hydrolysis, the AAC of wrinkled pea starch (64.0%) decreased slowly reaching a value of 26.0% after 20 days whereas the AAC of smooth pea starch (33.0%) decreased rapidly reaching a value of 5.0% after 12 days. This was in marked contrast to changes in AAC during α-amylolysis of smooth pea (Fig. 4-8c) and wrinkled pea starch (Fig. 4-8e). This difference can be explained as follows. In acid hydrolysis, amorphous domains are more rapidly hydrolyzed than crystalline domains (Hoover, 2000),

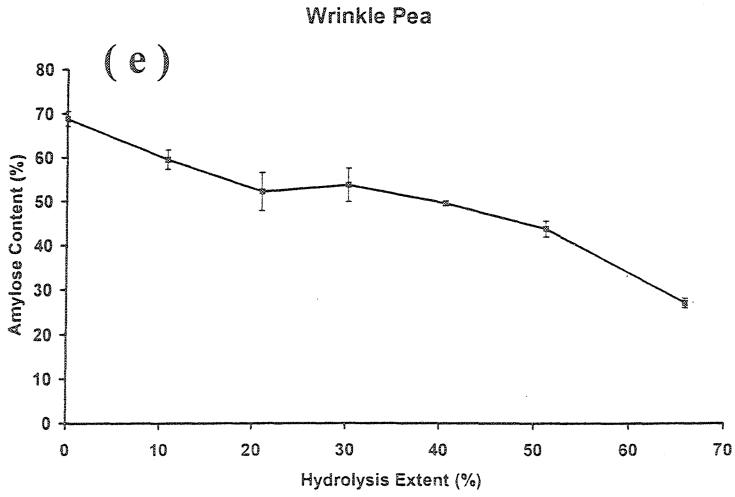
Figure 4-8 Apparent amylose content of legume starches at different time periods of α-amylase hydrolysis.


Black Bean
(a)
Black Jack
CDC Nighthawk
Amylose Content(%)
Hydrolysis Extent(%)


Pinto Bean
(b)
Othello
Sierra
Amylose Content (%)
Hydrolysis Extent (%)


Smooth Pea
(c)
CDC Sonata
CDC Mozart
Amylose Contlent (%)
Hydrolysis Extent (%)


Lentil
(d)
CDC Robin
CDC Redwing
Amylsoe Content(%)
Hydrolysis Extent(%)


Wrinkle Pea
(e)
Amylose Content (%)
Hydrolysis Extent (%)

whereas both amorphous and crystalline domains are hydrolyzed simultaneously during α-amylase hydrolysis (Lauro *et al.*, 1999). Thus, certain proportions of amylose chains in wrinkled pea starch are closely associated with each other, forming crystalline regions, and/or are co-crystallized with amylopectin. Hence, acid hydrolysis would be much slower than α-amylase hydrolysis.

4.9 Thermal properties of hydrolyzed starch residues

The gelatinization parameters of native and control starches and hydrolyzed residues are presented in Table 4-7. In general, gelatinization transition temperatures (T_o, T_p, T_c) increased slightly on hydrolysis. The extent of this increase was nearly of the same order of magnitude in all starches. However, increases in T_o, T_p and T_c were evident only for *CDC Mozart* and C*DC Robin* (Table 4-7). *Sierra, CDC Redwing* and *CDC Sonata* showed increases only in T_o, Tp and T_p & T_c, respectively (Table 4-7). Whereas, *Black Back, CDC Nighthawk* and *Othello* showed increases only in T_o and T_c (Table 4-7). In all starches, ΔH decreased on hydrolysis and the extent of this decrease was nearly the same for all starches (Table 4-7). The decrease in ΔH on hydrolysis reflects disorganization of amylopectin chains involved in double helical associations within the crystalline domains of the granule. Since T_o, T_p and T_c reflect crystallite melting, a decrease in ΔH should have theoretically resulted in a corresponding decrease in T_o, T_p and T_c. Thus, the increase in T_o, T_p and T_c on hydrolysis support my earlier postulation that hydrolyzed amylose chains retrograde, forming crystallites. The decrease in ΔH on hydrolysis suggests that the crystallites formed due to amylose retrogradation are not involved in double helical associations.

I postulate that the crystallites formed by amylose retrogradation probably differ in number and size among the hydrolyzed legume starches. This would explain why increases in T_o, T_p and T_c on hydrolysis occur only in some starches, whereas in others only one or two of the above

Table 4-7

DSC parameters[1] of α-amylase hydrolyzed legume starch residues

Starch source & cultivar		To[2](°C)	Tp(°C)	Tc(°C)	Tc - To(°C)	ΔH[3](mJ/mg)
Black bean						
Black Jack						
native		61.0 ± 0.2^{b}	70.9 ± 0.3^{a}	81.2 ± 0.3^{b}	20.3 ± 0.5^{a,b}	11.2 ± 0.1^{a}
control[4]		60.7 ± 0.3^{b}	71.3 ± 0.3^{a}	81.4 ± 0.4^{b}	20.8 ± 0.5^{a}	11.4 ± 0.2^{a}
hydrolyzed	28.3%	62.6 ± 0.2^{a}	70.8 ± 0.2^{a,b}	82.5 ± 0.4^{a}	19.9 ± 0.4^{a,b}	10.2 ± 0.2^{b}
	55.7%	63.5 ± 0.6^{a}	70.2 ± 0.1^{b}	82.5 ± 0.3^{a}	19.0 ± 0.7^{b}	10.0 ± 0.3^{b}
CDC Nighthawk						
native		65.7 ± 0.3^{e}	74.9 ± 0.4^{e}	86.7 ± 0.2^{e}	21.0 ± 0.1^{e}	12.2 ± 0.6^{e}
control[4]		65.6 ± 0.5^{e}	75.0 ± 0.3^{e}	86.3 ± 0.4^{e}	20.6 ± 0.4^{e}	12.1 ± 0.3^{e}
hydrolyzed	24.6%	66.5 ± 0.4^{e,f}	75.6 ± 0.1^{e}	86.7 ± 0.1^{e}	20.1 ± 0.5^{e}	10.3 ± 0.7^{f}
	58.8%	67.2 ± 0.6^{f}	75.4 ± 0.5^{e}	88.2 ± 0.4^{f}	21.0 ± 0.6^{e}	11.0 ± 0.5^{e,f}
Pinto bean						
Othello						
native		64.5 ± 0.2^{g}	76.5 ± 0.6^{g}	88.8 ± 0.3^{g}	24.3 ± 0.4^{g}	12.2 ± 0.2^{g}
control[4]		64.5 ± 0.4^{g}	76.5 ± 0.3^{g}	88.8 ± 0.4^{g}	24.3 ± 0.6^{g}	12.4 ± 0.2^{g}
hydrolyzed	18.1%	66.5 ± 0.3^{h}	77.1 ± 0.2^{g}	89.9 ± 0.2^{h}	23.4 ± 0.3^{g}	10.7 ± 0.3^{h}
	41.2%	66.9 ± 0.3^{h}	77.0 ± 0.2^{g}	90.2 ± 0.2^{h}	23.4 ± 0.2^{g}	10.2 ± 0.4^{h}
Sierra						
native		63.3 ± 0.2^{l}	70.9 ± 0.2^{l}	85.1 ± 0.7^{l}	21.8 ± 0.5^{l}	12.9 ± 0.1^{l}
control[4]		63.5 ± 0.4^{l}	70.8 ± 0.4^{l}	85.1 ± 0.2^{l}	21.6 ± 0.4^{l,m}	12.9 ± 0.1^{l}
hydrolyzed	29.0%	64.3 ± 0.2^{m}	70.7 ± 0.1^{l}	85.7 ± 0.6^{l}	21.5 ± 0.8^{l,m}	10.6 ± 0.2^{m}
	46.1%	65.6 ± 0.2^{n}	71.1 ± 0.2^{l}	86.0 ± 0.3^{l}	20.4 ± 0.3^{m}	10.9 ± 0.4^{m}

1. For each cultivar, data with the same superscript in the same column are not significantly different ($P < 0.05$). The data represent the mean ± SD of three determinations.
2. To, Tp, Tc indicate the onset, peak and conclusion temperature of gelatinization, respectively. Tc - To indicates the temperature range of gelatinization.
3. ΔH, Gelatinization enthalpy
4. Treated without α-amylase but subjected to the same experimental conditions.

Table 4-7

DSC parameters[1] of α-amylase hydrolyzed legume starch residues(cont'd)

Starch source & cultivar		To[2](°C)	Tp(°C)	Tc(°C)	Tc - To(°C)	ΔH[3](mJ/mg)
Smooth pea						
CDC Sonata						
native		60.1 ± 0.2^{q}	66.0 ± 0.2^{q}	76.4 ± 0.2^{q}	16.3 ± 0.4^{q}	10.1 ± 0.3^{q}
control[4]		60.2 ± 0.4^{q}	66.0 ± 0.2^{q}	76.3 ± 0.3^{q}	16.2 ± 0.6^{q}	10.1 ± 0.3^{q}
hydrolyzed	17.0%	58.2 ± 0.4^{r}	65.8 ± 0.2^{q}	76.7 ± 0.1^{q}	18.5 ± 0.4^{r}	8.5 ± 0.8^{r}
	47.8%	60.2 ± 0.5^{q}	67.1 ± 0.6^{r}	78.4 ± 0.2^{r}	18.2 ± 0.6^{r}	8.8 ± 0.2^{r}
CDC Mozart						
native		60.0 ± 0.4^{s}	66.6 ± 0.1^{s}	77.5 ± 0.4^{s}	17.5 ± 0.7^{s}	10.8 ± 0.5^{s}
control[4]		60.2 ± 0.5^{s}	66.7 ± 0.2^{s}	77.3 ± 0.3^{s}	17.2 ± 0.6^{s}	11.0 ± 0.3^{s}
hydrolyzed	17.3%	59.4 ± 0.3^{s}	67.2 ± 0.3^{s}	77.6 ± 0.3^{s}	18.2 ± 0.5^{s}	9.2 ± 0.3^{t}
	47.0%	61.2 ± 0.1^{t}	68.5 ± 0.3^{t}	80.9 ± 0.4^{t}	19.7 ± 0.3^{t}	$10.1 \pm 0.4^{s,t}$
Lentil						
CDC Redwing						
native		63.9 ± 0.1^{u}	70.6 ± 0.1^{u}	80.1 ± 0.9^{u}	16.2 ± 1.0^{u}	11.3 ± 0.3^{u}
control[4]		63.9 ± 0.2^{u}	70.5 ± 0.3^{u}	80.6 ± 0.5^{u}	16.7 ± 0.5^{u}	11.2 ± 0.2^{u}
hydrolyzed	29.0%	64.2 ± 0.4^{u}	71.3 ± 0.2^{v}	79.8 ± 0.3^{u}	15.6 ± 0.1^{u}	9.6 ± 0.1^{v}
	52.6%	64.3 ± 0.3^{u}	71.8 ± 0.1^{v}	81.1 ± 0.3^{u}	16.9 ± 0.1^{u}	9.6 ± 0.5^{v}
CDC Robin						
native		$61.1 \pm 0.2^{w,x}$	67.7 ± 0.1^{w}	77.3 ± 0.3^{w}	16.2 ± 0.2^{w}	9.9 ± 0.1^{w}
control[4]		60.9 ± 0.4^{w}	67.6 ± 0.3^{w}	77.1 ± 0.4^{w}	16.2 ± 0.5^{w}	9.9 ± 0.2^{w}
hydrolyzed	21.4%	61.7 ± 0.3^{x}	68.3 ± 0.2^{x}	78.0 ± 0.3^{w}	16.2 ± 0.2^{w}	9.1 ± 0.3^{x}
	61.9%	63.9 ± 0.2^{y}	70.7 ± 0.1^{y}	81.1 ± 0.4^{x}	17.3 ± 0.5^{x}	8.8 ± 0.2^{x}
Wrinkled pea						
native[5]		—	—	—	—	—
control[4]		—	—	—	—	—
hydrolyzed	22.1%	—	—	—	—	—
	53.5%	—	—	—	—	—

1. For each cultivar, data with the same superscript in the same column are not significantly different ($P < 0.05$). The data represent the mean ± SD of three determinations.
2. To, Tp, Tc indicate the onset, peak and conclusion temperature of gelatinization, respectively. Tc - To indicates the temperature range of gelatinization.
3. ΔH, Gelatinization enthalpy
4. Treated without α-amylase but subjected to the same experimental conditions.
5. Gelatinization characteristics were not detected within the temperature range 25°C to 145°C.

parameters increase on hydrolysis (Table 4-7). Furthermore, the absence of an endotherm in hydrolyzed wrinkled pea starch (Table 4-7) also suggests a large extent of amylose retrogradation during hydrolysis. This seems plausible, since the swelling factor of 58.5% for hydrolyzed wrinkled pea starch residue (1.8) was lower than that of its native counterpart (3.4).

Summary and Conclusions

1. This study has shown that the granular morphology, relative crystallinity, X-ray pattern ('C' type), extent of starch damage, 'B' polymorphic content and composition differed only marginally among black bean, pinto bean, smooth pea and lentil starches.

2. Swelling factor, amylose leaching and differential scanning calorimetry measurements showed that the extent of starch chain interactions within the amorphous and crystalline domains of the native granule were more pronounced in pinto bean than in black bean, lentil and smooth pea starches. Wrinkled pea differed from the other legume starches in exhibiting a higher extent of starch damage, a higher content of bound lipids, a different X-ray pattern ('B' type), lower relative crystallinity, different granule shapes and sizes, a highly disrupted crystalline structure and strong interaction between amylose chains.

3. The rate and extent of α-amylolysis of black bean, pinto bean, smooth pea and lentil starches were mainly influenced by the interplay of : 1) the magnitude of interaction between starch chains within the amorphous domains of the native granule; and 2) extent to which hydrolyzed amylose chains interact with each other during the time course of hydrolysis. However, in wrinkled pea starch, in addition to the above two factors, a disrupted crystalline structure (influenced by its higher amylose content and longer amylopectin chain length), a higher 'B' polymorphic content and a higher extent of starch damage were also causative factors influencing the rate and extent of hydrolysis. Differences in hydrolysis between cultivars were evident only in black bean and lentil starches.

Directions for Future Research

1. A study of the fine structures of amylose and amylopectin from the legume starches used in this study would give a deeper insight into how starch chains influence α-amylolysis.

2. Application of TEM (Transmission Electron Microscopy) and AFM (Atomic Force Microscopy) may reveal more information on changes in ultrastructure and surface morphology during enzymatic hydrolysis.

3. Since legume starches are generally resistant to α-amylase attack and exhibit a low glycermic index *in vivo*, which is considered beneficial to human health, it is worthwhile to explore their potential as resistant starch (RS) or slowly digestible starch (SDS) related food products.

4. Legume starches have very limited applications in the food industry, partly due to their unattractive properties (e.g. high retrogradation rates). Therefore, further research (i.e. physical and chemical modifications) aimed at to improving their performance would be useful.

Publications

1. **Zhou, Y.** and Hoover, R. (2003) Relationship between α-amylase degradation and the structure and physicochemical properties of legume starches (in review).

2. Hoover, R. and **Zhou, Y.** (2003) In vitro and in vivo hydrolysis of legume starches by α-amylase and resistant starch formation in legumes – a review. *Carbohydr. Polym.*, 54: 401 – 417.

3. Hoover, R., Smith, C., **Zhou, Y.** and Ratnayake, R.M.W.S. (2003) Physicochemical properties of Canadian oat starches. *Carbohydr. Polym.*, 52: 253-261.

Presentations

1. Poster presentation, 12th IUFoST conference, Chicago, IL. USA, Jul.16 – 20, 2003.

Honors and Awards

1. Barrowman Biochemistry Graduate Travel Award, Year 2003-2004.

2. Title of "Fellow of the School of Graduate Studies" in recognition of outstanding achievement in graduate program, Year 2002-2003

3. Graduate Fellowship, Graduate School, Memorial University of Newfoundland, St. John's, NL, Canada (from Dec. 2002 to Dec. 2003).

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Appendices

Figure A-1 Standard curve for amylose determination

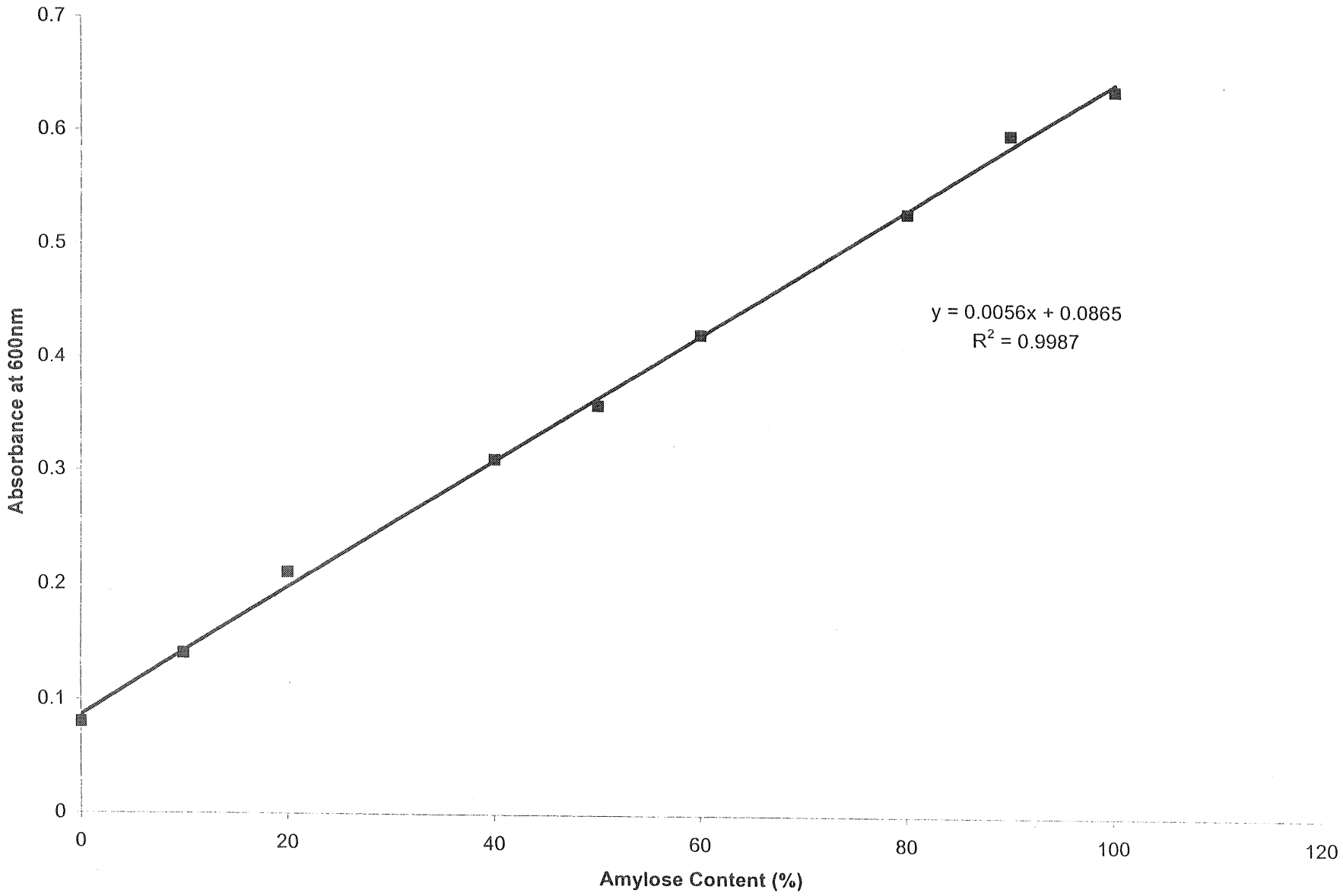

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance at 600nm
y = 0.0056x + 0.0865
R2 = 0.9987
0
20
40
60
80
100
120
Amylose Content (%)

Figure A-2 Standard curve for reducing sugar determination as maltose at 540nm

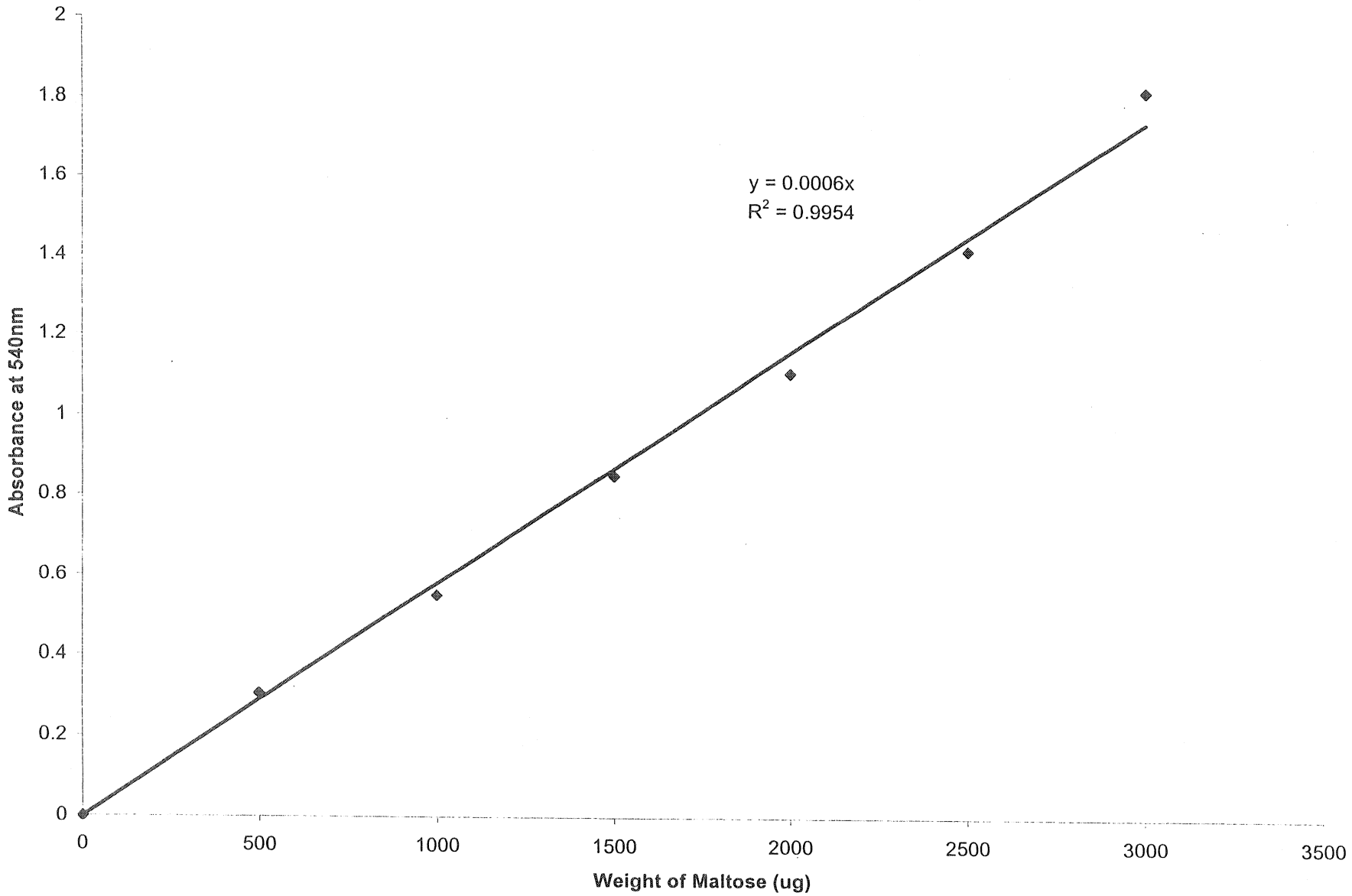
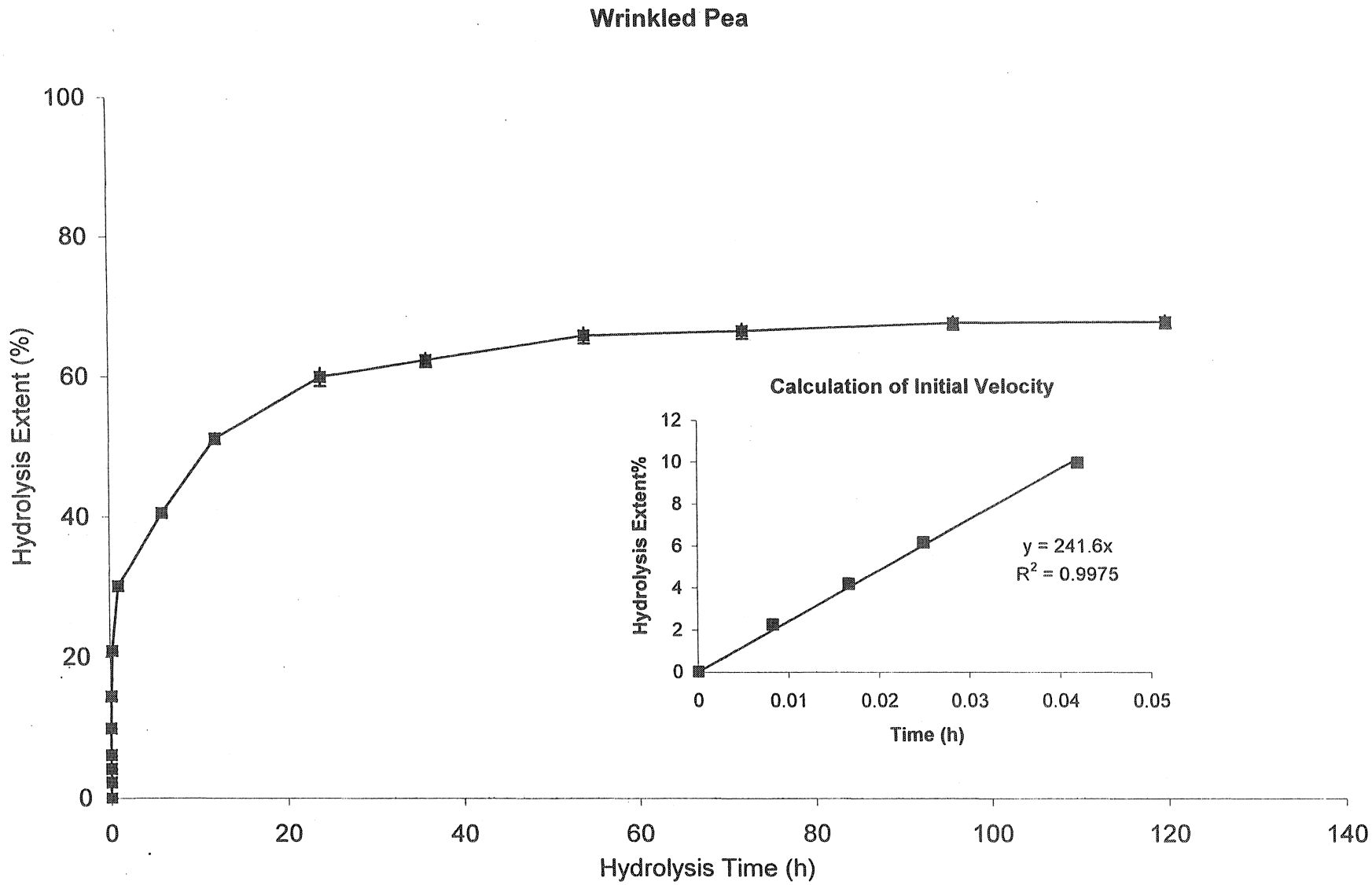
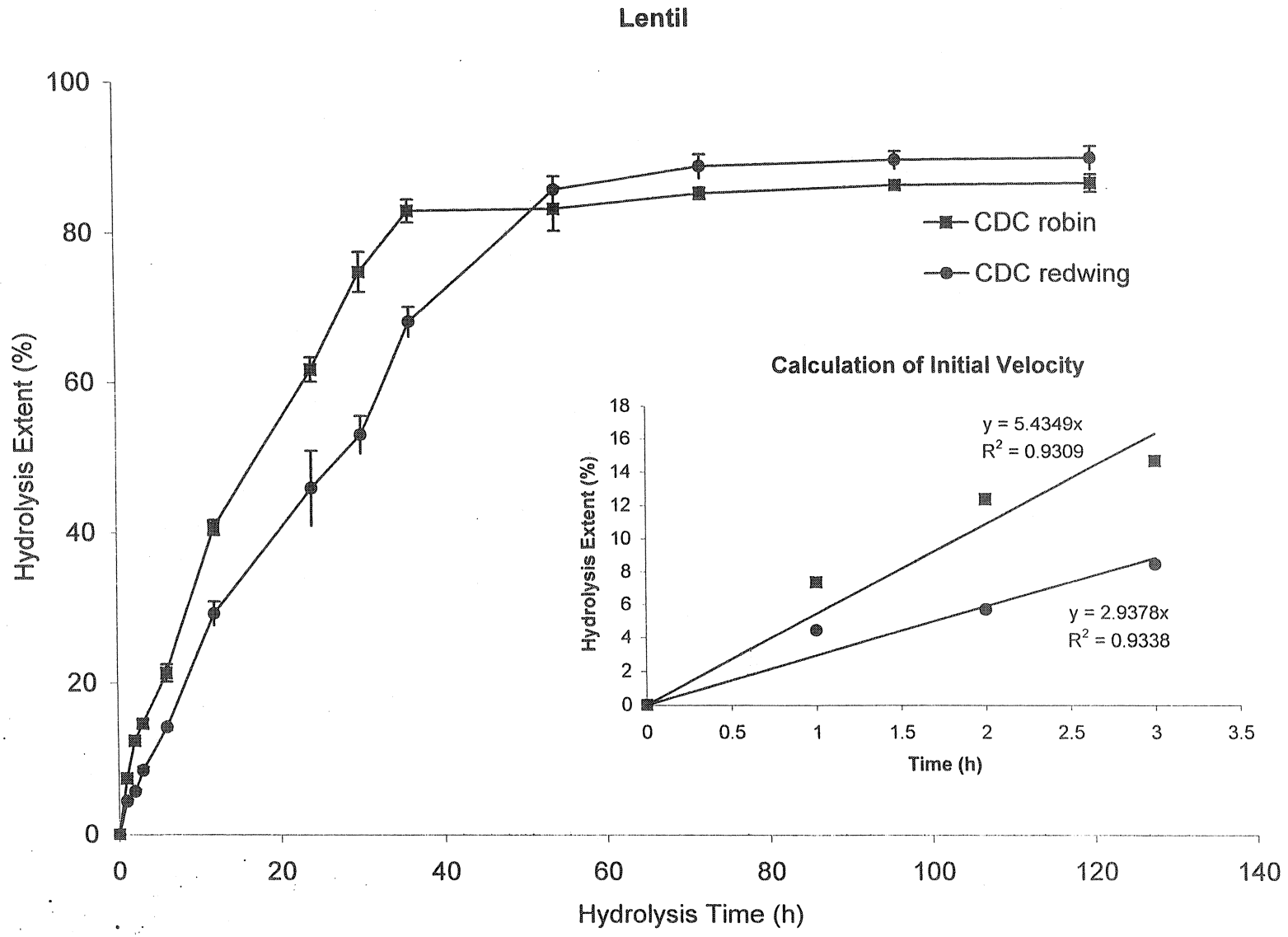
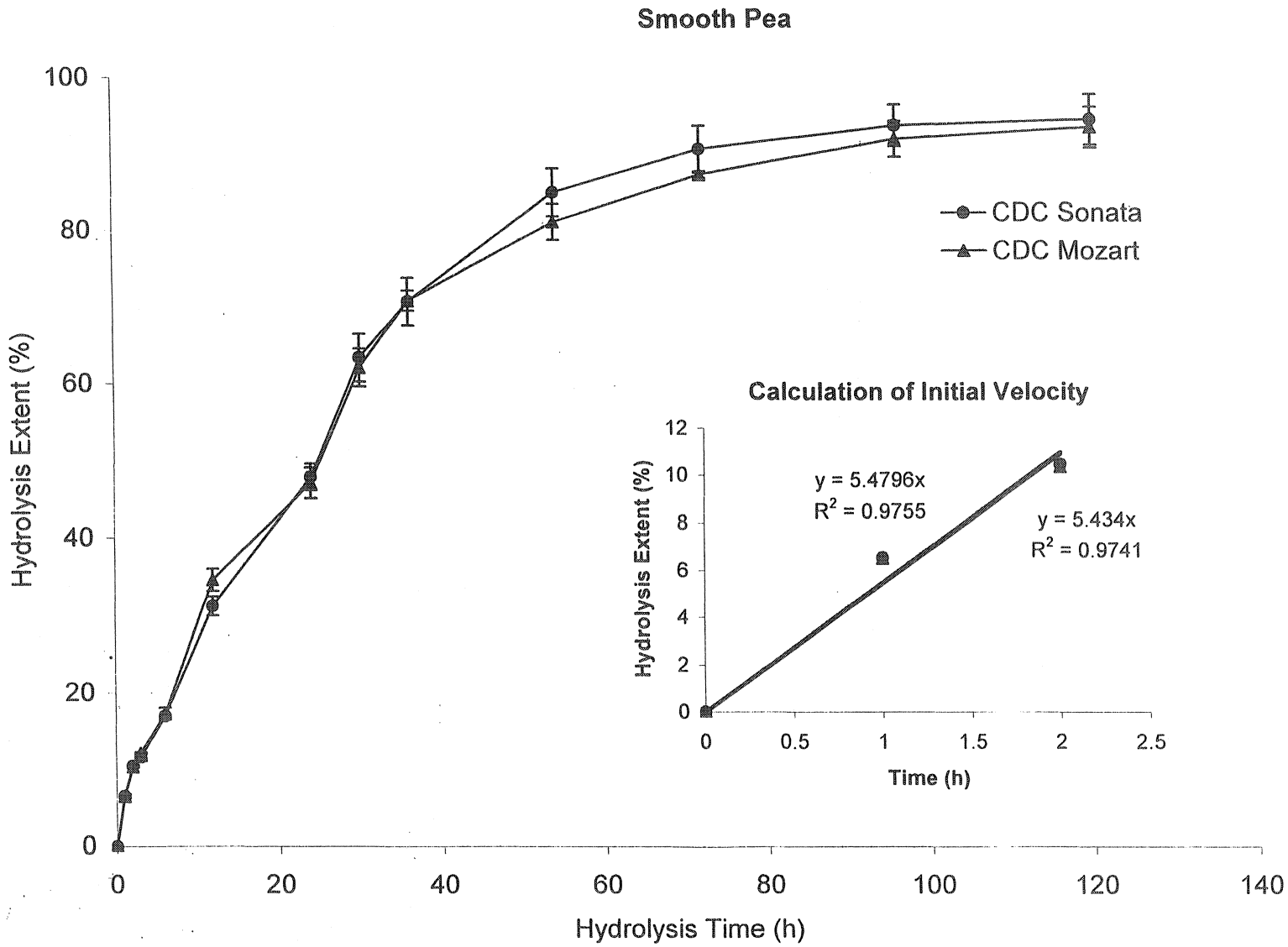
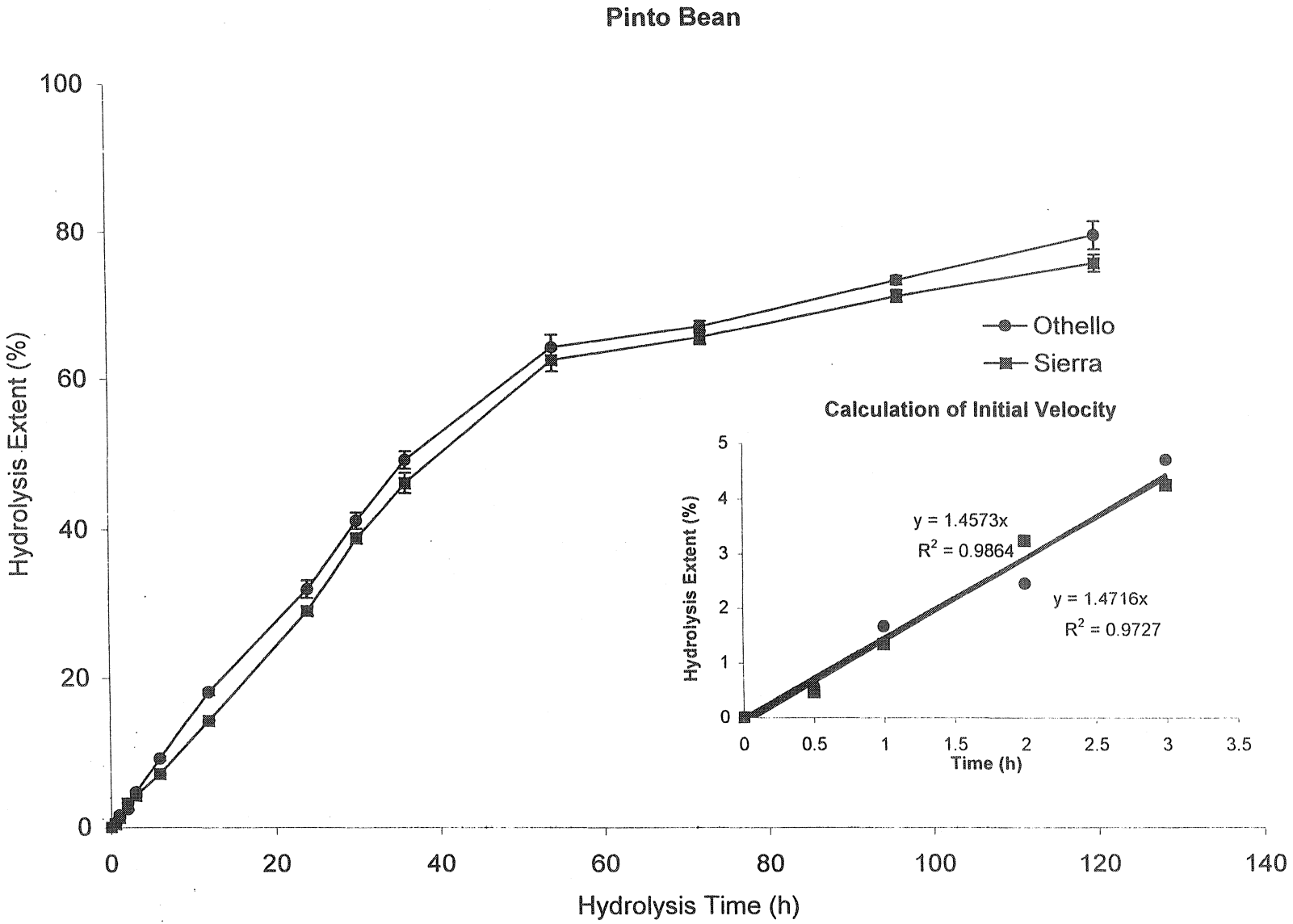

y = 0.0006x
R2 = 0.9954
Absorbance at 540nm
Weight of Maltose (ug)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
500
1000
1500
2000
2500
3000
3500

Figure A-3 Schematic illustration for initial velocity determination


Wrinkled Pea
Hydrolysis Extent (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Hydrolysis Time (h)
Calculation of Initial Velocity
Hydrolysis Extent%
12
10
8
6
4
2
0
0
0.01
0.02
0.03
0.04
0.05
Time (h)
y = 241.6x
R2 = 0.9975


Lentil
Hydrolysis Extent (%)
Hydrolysis Time (h)
CDC robin
CDC redwing
Calculation of Initial Velocity
Hydrolysis Extent (%)
Time (h)
y = 5.4349x
R2 = 0.9309
y = 2.9378x
R2 = 0.9338


Smooth Pea
Hydrolysis Extent (%)
Hydrolysis Time (h)
CDC Sonata
CDC Mozart
Calculation of Initial Velocity
Hydrolysis Extent (%)
Time (h)
y = 5.4796x
R2 = 0.9755
y = 5.434x
R2 = 0.9741


Pinto Bean
Hydrolysis Extent (%)
Hydrolysis Time (h)
Othello
Sierra
Calculation of Initial Velocity
Hydrolysis Extent (%)
Time (h)
y = 1.4573x
R2 = 0.9864
y = 1.4716x
R2 = 0.9727

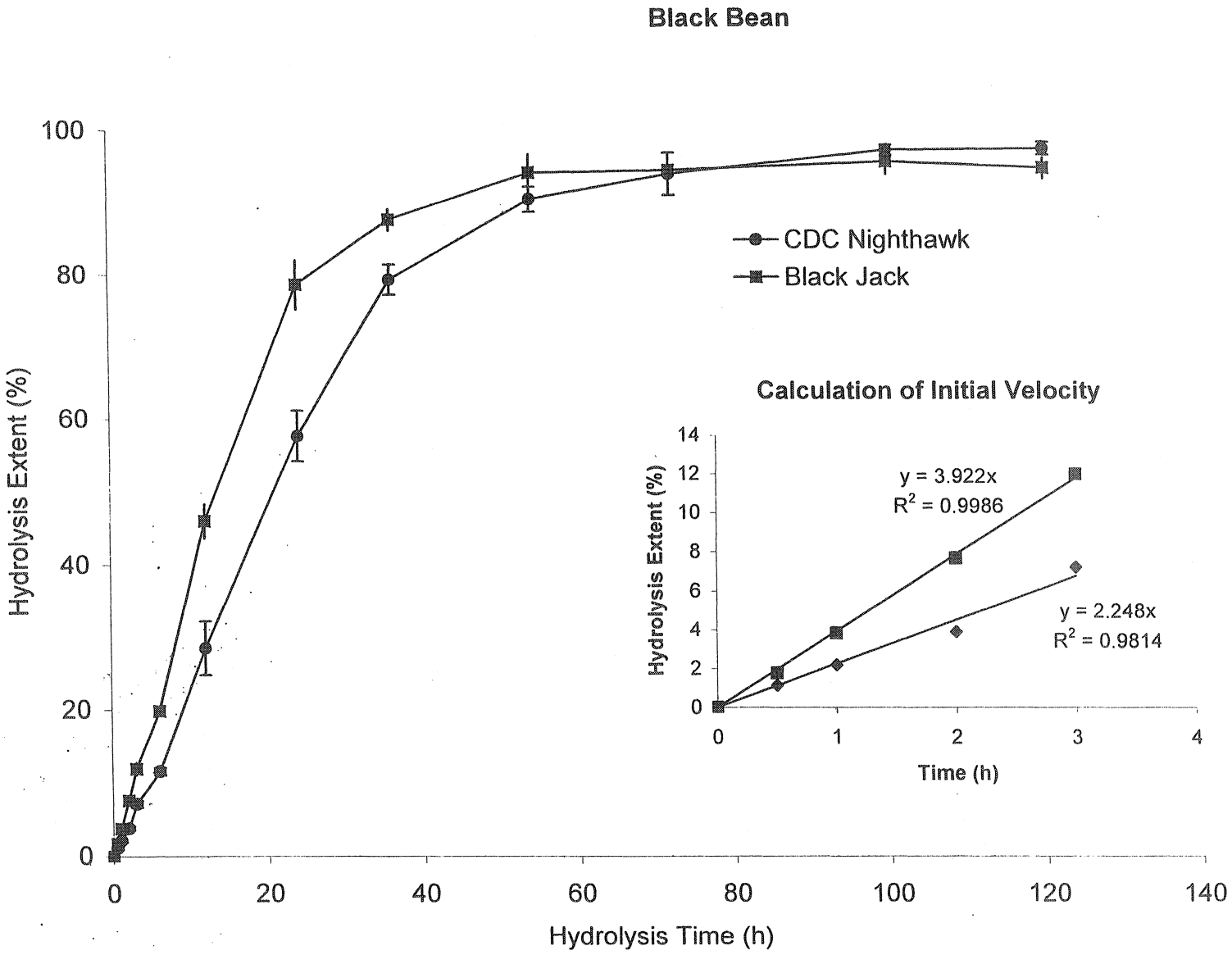

Black Bean
Hydrolysis Extent (%)
Hydrolysis Time (h)
CDC Nighthawk
Black Jack
Calculation of Initial Velocity
Hydrolysis Extent (%)
Time (h)
y = 3.922x
R2 = 0.9986
y = 2.248x
R2 = 0.9814

Figure A-4 Schematic illustration for gelatinization parameters determination

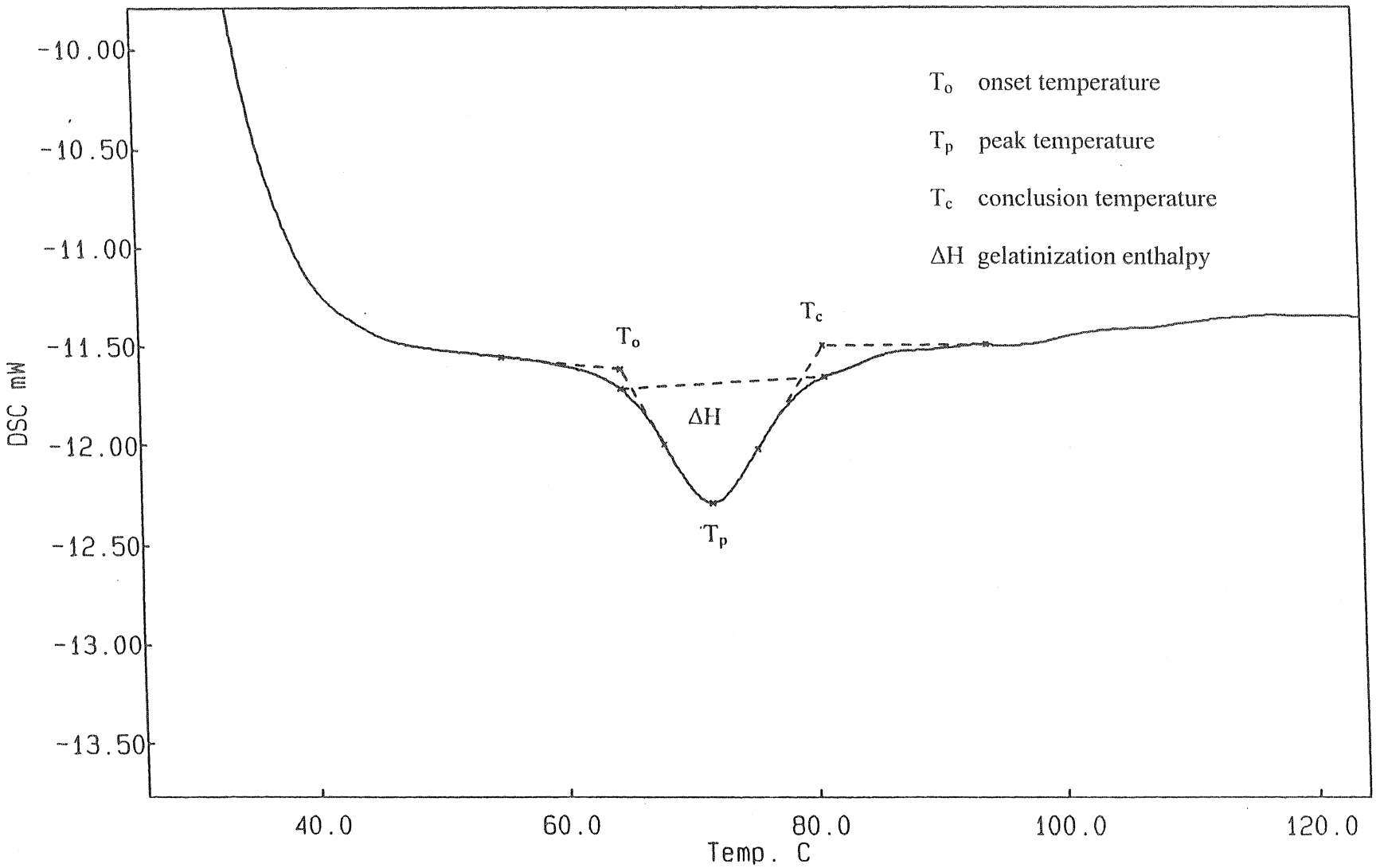

-10.00
-10.50
-11.00
-11.50
-12.00
-12.50
-13.00
-13.50
DSC mW
40.0
60.0
80.0
100.0
120.0
Temp. C
T_o onset temperature
T_p peak temperature
T_c conclusion temperature
ΔH gelatinization enthalpy
T_o
T_c
ΔH
T_p

Figure A-5 Schematic illustration for relative crystallinity determination

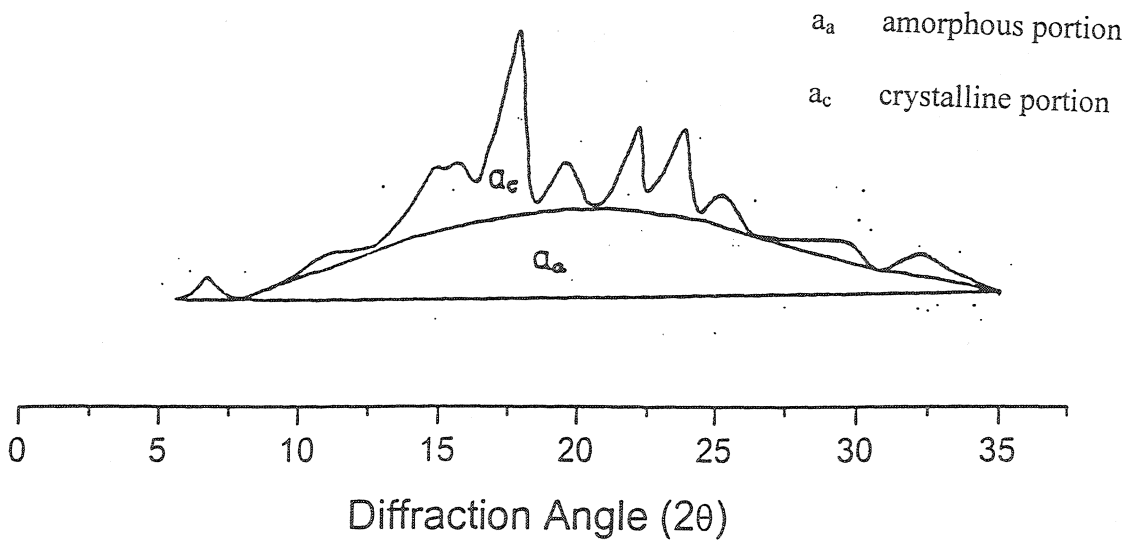
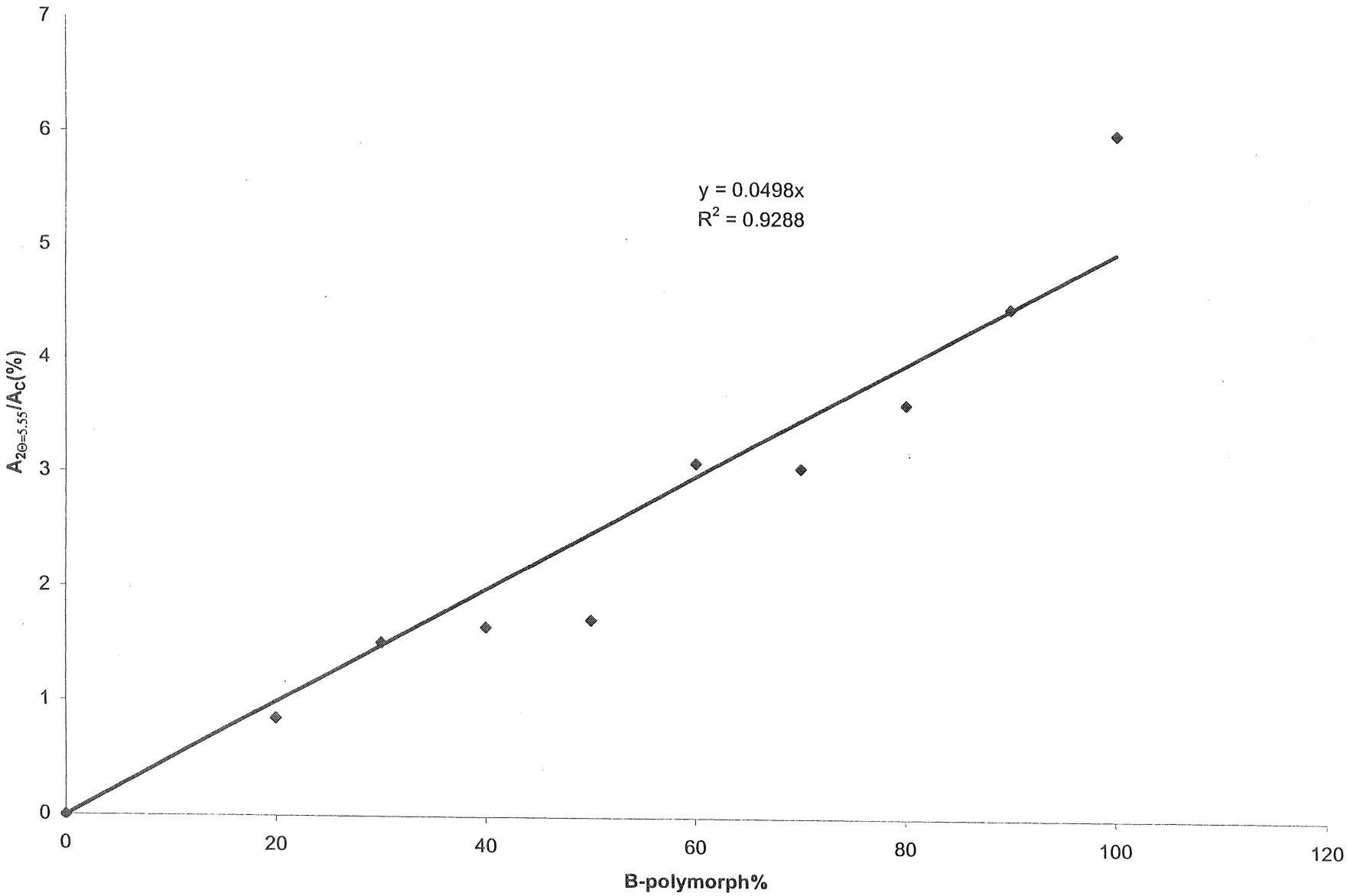

a_a amorphous portion
a_c crystalline portion
a_c
a_a
0
5
10
15
20
25
30
35
Diffraction Angle (2θ)

Figure A-6 Standard curve for 'B' polymorphic content determination


y = 0.0498x
R2 = 0.9288
A2θ=5.55/AC(%)
B-polymorph%
0
1
2
3
4
5
6
7
0
20
40
60
80
100
120